

The Potential Role of Kv3.3 in Chemotherapy-Induced Neuropathy

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Emily Pfahl

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Approved by:

Dr. Timothy Cope
School of Biological Science
Georgia Institute of Technology

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ABSTRACT

Oxaliplatin (OX) is a widely used chemotherapy compound used in the treatment of colorectal cancers (CRCs). Patients treated with OX often exhibit severe side effects, including motor dysfunction and imbalance, potentially influenced by the inability of sensory neurons, including muscle spindle afferents (MSAs), to repetitively fire, which is needed to properly encode information about limb movement. Even though OX is prescribed in a majority of CRCs, it is currently unknown how the compound causes the aforementioned side effects. The aim of the present study was to determine if OX acts through modification of the voltage-gated potassium channel Kv3.3, which is hypothesized to promote repetitive firing. To test this hypothesis, the soleus nerve and muscle were isolated from control mice. A series of ramp and triangular stretches were applied to the muscles, and afferent firing responses were recorded. A synthetic Kv3.3-knockdown line of mice was created using Kv3.3 siRNA (ThermoFischer Scientific). The soleus nerve and muscle were isolated from these mice, the same stretches were applied to the muscle, and MSA recordings were taken and compared to the control MSA responses. Preliminary data suggest that afferent responses to stretch are altered in the Kv3.3-knockdown mice, but as of this time, not enough data has been collected to make statistically significant claims. Future work will focus on collecting enough Kv3.3-knockdown data to perform statistical analyses on the data, as well as on performing immunohistochemical (IHC) staining of tissue from knockdown animals to ensure silencing of Kv3.3.

INTRODUCTION

Compared to other cancer types, colorectal cancers (CRCs) cause the second-highest number of deaths annually with a mortality rate of 13.9%. Though the overall incidence of CRCs is declining, the number of afflicted individuals in the United States is projected to reach nearly 150,000 in 2020 alone [1]. Oxaliplatin (OX) is a platinum-based chemotherapy compound that inhibits DNA replication and transcription [2] and is commonly used in the treatment of CRCs. Though successful, treatment with OX has been shown to have multiple debilitating side effects, including sensory neuropathy and ataxia, which decrease quality of life and can compound with aging [3].

Because of the complexity of OX's interactions with cells, the mechanism through which OX works to affect motor functioning and cause neuropathy is unknown. Previous studies have shown that OX treatment in rats without cancer disrupts muscle spindle afferent (MSA) firing [4], suggesting that the source of the negative side effects of OX may lie in disruption of the ion channels, i.e. channelopathy in the terminal neural receptor endings. Because of their role in sustained repetitive firing, channels responsible for sodium persistent inward current (NaPICs) were examined in a previous study. While it was shown that NaPIC antagonists affected MSA firing similarly to how OX affects firing, the NaPIC channels were not downregulated in muscle spindles [5]. However, a study our lab conducted last year points to another potential channel implicated in OX-mediated neuropathy. Genomic analysis showed that rats treated with OX exhibited, along with aberrant afferent firing, a significant downregulation of the 3.3-type potassium voltage-gated channel (Kv3.3) [5].

It has long been hypothesized that Kv3.3 plays a role in repetitive firing. Because Kv3.3 was only discovered in the muscle spindles within the last year [5], no studies to date have

examined the role Kv3.3 plays in spindle response to stretch, or how knocking out the channel can affect firing rate. Kv3.3 has been shown to be present in cerebellar Purkinje cells [6] and downregulation of the channels is consistently seen in cases of spinocerebellar ataxia [7]. Individuals with the disease exhibit motor disabilities as well as the inability of neurons to repetitively fire [7]. This study will focus on explicitly determining the function(s) of the Kv3.3 channel in the muscle spindles and elucidate if Kv3.3-knockdowns have the same inability to sustain repetitive firing as seen in OX-treated animals. We expect that, because of Kv3.3's proposed role in repetitive firing in Purkinje neurons, as well as the downregulation of the channels in the spindles of rats treated with OX, Kv3.3-knockdown mice will result in MSAs that are unable to sustain repetitive firing.

LITERATURE REVIEW

As stated previously, OX is a chemotherapy drug commonly used to treat CRCs; this is because of its high incidence of cancer-free survival when combined with other treatments [8]. Treatment with OX, however, has several debilitating side effects, including sensory neuropathy, which is seen in up to 80% of treated patients [9, 10]. Sensory neuropathy is often characterized by paresthesia, a prickling sensation in the limbs, as well as motor ataxia, the inability to coordinate movements, and is caused by altered proprioceptive inputs [11]. While it has been shown that OX treatment results in abnormal firing of proprioceptive afferents [5], the cause of the aberrant firing is not currently known. In a recent study conducted by our lab, it was found that the voltage-gated potassium channel Kv3.3 was significantly downregulated in rats with cancer that were treated with OX [5]. Kv3.3-knockout animals have previously been shown to exhibit similar symptoms as OX-treated animals with cancer, such as peripheral neuropathy and ataxia [12]. Based on our recent finding that the Kv3.3 channels in OX-treated animal cancer models were downregulated, coupled with the known functions of Kv3.3, impairment of the channel is a likely candidate for the method through which OX works to create the associated neuromuscular side effects.

The fibers comprising muscles can be divided into two types: extrafusal and intrafusal. Gamma motor neurons, receiving cortical input, innervate the intrafusal fibers [13]. Specialized proprioceptive neurons called muscle spindle afferents (MSAs) wrap around the intrafusal fibers. When the muscle spindle receptors are stretched, either by stimulation of the gamma motor neurons and subsequent intrafusal fiber contraction or whole-muscle stretch, mechanosensitive ion channels in the spindles' primary endings open. The subsequent influx of cations results in the production of a receptor potential, which depolarizes the sensory terminal.

This causes various voltage-gated ion channels to open, which transduces the receptor potential into an action potential in the primary afferent [14]. There are three types of specialized sensory receptors in the muscles: types Ia and II are muscle spindles, and type Ib are Golgi tendon organs (GTOs). Muscle spindles are sensory receptors that encode muscle length and stretch velocity, while GTOs are found in the tendons and encode tension [14]. During electrophysiological recording, the type of receptor-associated afferent can be deduced using three criteria: firing during the rising phase of an isometric twitch, entrainment to high-frequency vibrations, and firing at the onset of a rapid stretch [15]. The muscle spindles have a characteristic pause in firing during isometric twitch contractions, while GTOs only fire during the rising phase of the contractions. Types Ia and II spindle afferents can further be distinguished by response to high frequency vibrations: type Ia afferents exhibit perfect entrainment, while type II afferents do not. Furthermore, type Ia afferents have a high instantaneous firing frequency at the onset of dynamic stretch, while type II afferents typically do not [15]. Type Ib GTO afferents can be distinguished from types Ia and II spindle afferents during response to ramp stretches, as type Ib afferents will exhibit an increased firing rate during the rising phase of the stretch [15].

It has been hypothesized that OX targets the ion channels in the muscle spindles to produce the seen side effects. In a previous study, the channels responsible for persistent-inward sodium current (NaPIC) were studied in rats treated with OX [16]. A possible role of NaPIC in MSA dysfunction was suggested by the observation that a pharmaceutical antagonist to NaPIC recreated the impairment in firing seen with OX. However, further study showed that NaPIC channels were not downregulated in the spindles, a finding that redirected attention to other ion channels as possible contributors to OX-induced sensory neuropathy [16]. A subsequent study of OX-treated animals examined the relative concentrations of other ion channels and proteins

known to be present in the proprioceptors. These included VGLUT1 (mechanosensitivity); Kv1.1 (action potential encoding); NaV1.1, NaV1.6, and NaV1.7 (signal amplification); and ASIC2 (mechanotransduction). None of these proteins was found to be downregulated, although post-translational defects could still lead to impairment of channels without downregulation [5].

Though various types of voltage-gated sodium and potassium ion channels have been discovered in the muscle spindles [14], it was possible that a channel yet to be identified in the muscle spindles was implicated in the neuromuscular defects. Recently, our lab tested for the presence of the voltage-gated potassium channel Kv3.3 in the spindles [5]. Previous studies demonstrated that mutations of the Kv3.3 channel are present in cases of spinocerebellar ataxia, a severe neurological condition that targets the Purkinje cells in the cerebellar cortex [17]. This condition is characterized by motor deficits similar to those seen in OX patients, and disruption of Purkinje cell repetitive firing similar to that in muscle spindles of OX animals [5, 18]. Our lab not only discovered that Kv3.3 channels are present in the muscle spindles, but that they are also significantly downregulated in animals treated with OX. Therefore, modification of the Kv3.3 channel could potentially be the cause of the motor deficits and irregular afferent firing seen in OX-treated animals [5]. Because of this finding, focus for the current study was directed towards Kv3.3 rather than VGLUT1, NaV1.1, NaV1.6, NaV1.7, and ASIC2.

Because of the recency of the discovery of Kv3.3 in the muscle spindles, further studies need to be conducted to determine if significant downregulation of the channels can cause the impairment of repetitive afferent firing seen in OX-treated animals. It is hypothesized that, because of Kv3.3's proposed role in repetitive firing, as well as the discovery that Kv3.3 is significantly downregulated in OX-treated animals, knocking down Kv3.3 will result in recreation of the impaired MSA firing seen with OX treatment. The current study will attempt to

address the current gap in knowledge by recording *in vitro* (as described by Franco et. al [19]) from soleus muscle sensory receptor afferents of both control and Kv3.3-knockdown mice. The Kv3.3 knockdowns will be created using siRNA to silence the RNA coding for the Kv3.3 channel. MSA responses to three stretch paradigms will be recorded: ramp-hold-release stretches with changing amplitudes and a constant velocity, ramp-hold-release stretches with changing velocities and a constant amplitude, and sets of three serial triangular stretches. If the knockdown animals are found to exhibit similar firing disruptions as seen in OX animals, this will provide a more complete picture of the way in which OX causes sensory neuropathy in cancer patients. Results can be applied to a potential therapeutic treatment for the debilitating OX-induced side effects. The following section details the methodology used to record spindle afferent responses to the different stretch paradigms, as well as how the data will be analyzed once more results are recorded.

METHODOLOGY

Animal Care

All procedures were approved by the Georgia Institute of Technology Institutional Animal Care and Use Committee. Adult male mice were held in clean cages with regular access to water and food in a temperature- and light-controlled environment. All animals were anesthetized using isoflurane prior to and during leg extraction and were subsequently euthanized via surgical removal of the heart.

Kv3.3 Knockdowns and Immunohistochemistry

Kv3.3 knockdowns were created using Kv3.3 siRNA (ThermoFisher Scientific). Conscious mice were injected with 150µl of siRNA approximately 48 hours prior to surgical isolation of the muscle. After removing the mouse's legs, the animal was euthanized via surgical removal of the heart and immediately perfused, and its kidneys, spinal cord, and brain were collected. These tissues, as well as the unused second leg and muscle preparation at the conclusion of the experiment, were then preserved in formalin for immunohistochemistry (IHC).

IHC will be conducted on tissue collected from each Kv3.3-knockdown mouse. The muscle preparation, leg, brain, and/or kidneys will be cut using a cryostat into 40µm-thick slices; the spinal cord will be cut into 30µm-thick slices. Slices will be frozen onto glass slides and then washed thrice for seven minutes with a solution of 0.01 M PBS and 0.3% triton. Each slide will then be covered with 400µL of blocking buffer for an hour, followed by 400µL of PBS/triton with primary Kv3.3 and VGLUT antibodies (each at a concentration of 1:200). Slides will then be incubated overnight. After 24 hours, the slides will be removed and washed thrice in the PBS/triton solution for seven minutes. The tissue will be covered with 400µL of PBS/triton with

secondary Kv3.3 and VGLUT antibodies (each at a concentration of 1:200) and incubate for two hours in complete darkness. Slides will be washed thrice with PBS/triton for seven minutes. H-1200 Vectashield with DAPI will be added and then slide covers will be placed over the tissue. Finally, tissues will be imaged via a Leica DM LFSA microscope to ensure there is no Kv3.3 staining in order to confirm siRNA knockdown.

Experimental Setup

In a beaker, 500 mL of Ringer's solution for muscles was made using the following procedure. 454.6 mL distilled water, 29.5 mL NaCl [2M], 13.1 mL NaHCO₃ [1M], 0.85 mL NaH₂PO₄ [1M], 0.58 mL KCl [3M], 0.35 mL MgSO₄ [1M], and 1.0 g glucose were combined in a beaker and infused with compressed oxygen gas. 1.0 mL CaCl₂ [1M] was added to this solution. A line of tubing was used to connect the beaker to a dissection dish; another line was connected from the dish to a pump and back to the beaker (Figure 1).

Surgical Procedure

Mice were first placed in a sealed container with an isoflurane-soaked pad for five minutes to ensure the animals had lost consciousness. The mice were then moved to a table and continued receiving anesthesia during removal of the legs. Curved forceps were applied at the lateral ankle joints and pulled rostrally to remove the skin. Legs were cut about halfway between the hip and knee joints and were immediately transferred to a dissection dish of Ringer's solution. One leg was pinned off to the side as an alternate in case the other leg was not viable, either from error during removal or during the dissection. The other leg was placed dorsal side up in the center of the dish and was oriented so the paw was facing the bottom of the dish.

The *soleus* muscle (SL) and nerve were surgically dissected using methodology adapted from Franco et al. (2014). First, the *biceps femoris* was cut down the midline using spring scissors and removed at the lateral sides of the leg. The *lateral gastrocnemius* (LG) and *medial gastrocnemius* (MG) were carefully separated using angled spring scissors. The LG tendon at the ankle joint was cut and tweezers were used to lift the muscle from the leg. Once the muscle was freed the LG tendon at the knee was cut and the muscle was removed. Next, the MG ankle tendon was severed, and the muscle was pulled away from the underlying *plantaris* muscle (PL) until the SL nerve was visible.

Using fine-point tweezers, the nerve was separated from the MG as close to its decussation from the sciatic nerve as possible. The MG knee tendon was then cut, and the muscle was removed from the leg. The SL nerve was then separated from the PL and cut as close to the sciatic nerve as possible. The PL ankle tendon was cut and pulled away from the underlying SL. Once the nerve was completely free from the PL, the PL knee tendon was severed and the muscle was removed. Silk sutures were tied around the SL tendons close to the muscle's origin (knee) and insertion (ankle). Cuts were made below and behind the ankle suture to free the bottom of the SL (a partial tarsus and connective tissue remained with the SL for stability). The SL was carefully peeled away from the tibia and fibula, and two more cuts were made above and behind the SL knee tendon (the patella, partial femur, and connective tissue remained with the SL for stability).

Mounting the Muscle and Recording

The bones and tissue at each end of the SL were pinned to a strip of Sylgard. The dissection dish was disconnected from the setup and replaced with a tissue bath. The SL muscle was transferred to the bath, and the suture at the knee tendon was tied to the bath's mounting

hook. The suture at the ankle tendon was tied to the arm of a force lever. Once secured, the muscle was unpinned from the Sylgard, and the Sylgard was removed from the bath (Figure 2). A 50- μ m glass electrode was attached to a micromanipulator, and a connected syringe was used to draw up Ringer's solution until approximately 1 mm of the inner silver wire was covered.

The electrode was positioned next to the nerve and the tip was suctioned into the electrode. To ensure the muscle and nerve were still viable, current was used to induce twitch contractions in the muscle. To determine if there were any active muscle afferents that could be recorded by the electrode, a command was sent using the program Spike2 for the force lever to administer a 100 Hz vibration. If there was no response to the vibration, the nerve was pushed out of the electrode and repositioned. Once there was a response to the vibration, the muscle underwent a series of ramp and three-triangular stretches. First, four ramp stretches were conducted for each of four different amplitudes with a constant velocity. Next, three or four three-triangular stretches were done. Finally, four ramp stretches were conducted for each of four different velocities with a constant amplitude (Table 1).

Classification of Afferents

Afferents were classified as Type Ia, Ib, or II based on response to ramp and triangular stretches. Types Ia and II are muscle spindle afferents (MSAs), and type Ibs are Golgi tendon organs (GTOs). The MSAs could be distinguished from GTOs because GTOs exhibit a characteristic increase in firing rate during the rising phase of a stretch. Types Ia and II could be differentiated by entrainment to high frequency vibrations: type Ias exhibit perfect entrainment, whereas type IIs do not. Additionally, type Ias have fast instantaneous firing frequencies at the onset of a dynamic stretch, whereas type IIs do not (Table II). This experiment analyzed data

exclusively from Ia afferents, as Ia responses typically are the easiest to discriminate; all other afferents were excluded from this analysis.

Statistical Analysis

MSAs were selected for analysis if strong firing rates in response to ramp stretches were observed and could be distinguished from noise. Only afferents concluded to be of Type Ia were further analyzed (see Classification of Afferents section). The data files were examined using the program Spike2 (version 8.02), which was used to discriminate spiking of the MSAs of interest from other afferents present and background noise. Extra spikes erroneously marked by the program or missed spikes were fixed by hand. A script was applied to each type of stretch to determine the timing of the first and last spikes, number of spikes during each part of the stretches, and firing rates. Two-way ANOVA tests will be used to examine differences in a variety of afferent response parameters between control and Kv3.3-knockdown animals.

DISCUSSION

There are four potential outcomes when comparing data collected from controls to the Kv3.3-knockdown mice. First, it is possible that the knockdowns will not have a significant change in the firing parameters when compared to the control data. It may be possible that my hypothesis, that knocking down Kv3.3 channels will recreate the MSA firing pattern seen in OX-treated animals, is incorrect. OX may alter the functioning of other types of channels found in the spindles, or it may alter a combination of Kv3.3 and other channels. It is also possible that OX acts on a different part of the nervous system (e.g., the motor neurons).

A second potential result would be that the knockdowns have no MSA activity in response to stretch. This result also suggests that OX may act on other channels, as OX-treated rats have been shown to still exhibit (abnormal) afferent firing in response to stretch. The third potential result is that knocking down Kv3.3 significantly reproduces the firing pattern seen in OX-treated animals. This result would provide the most support for my hypothesis; as Kv3.3 will be the only channel type knocked down, it would be very likely that OX interacts with this channel type alone to induce abnormal spindle afferent firing.

The fourth potential result is that the firing rate of Kv3.3-knockdown afferents differs in several parameters when compared to that of OX-treated afferents. Based on the data collected thus far, this seems to be the most likely outcome. While no data analysis has been performed yet, during MSA recording from Kv3.3-knockdown afferents, it was noted that firing rates appeared slower than that of controls. Another potential affected parameter is response to vibrations; it seemed as though type Ia-knockdown afferents rarely exhibited perfect entrainment during these stretches. Though there was not the observable drop-off in knockdown firing rate during ramp stretches that is seen in OX-treated animals, further analysis is needed to determine

if there is no statistical significance. If some parameters of knockdown MSA firing are significantly different from those of controls, but not the same as those of OX-treated animals, it would suggest that OX may alter a number of channels, which may or may not include Kv3.3.

CONCLUSION

The aim of this study was to determine what role, if any, the voltage-gated potassium channel Kv3.3 plays in OX-induced neuropathy. It is expected that synthetically knocking out these channels in the muscle spindles via siRNA will produce the pathological firing seen in animals treated with OX. If this result is seen, it suggests that OX exerts its effects by manipulating Kv3.3 channels, resulting in the inability to sustain repetitive firing. Therefore, Kv3.3 should be a channel of interest in future studies aiming to alleviate OX's neuropathic side effects. However, much future work is needed to assess the significance of any results. More afferent recordings (at least ten in total) are needed from Kv3.3 knockdowns to significantly compare responses to stretch between the knockdown and control mice. Furthermore, IHC will also need to be performed to show that the siRNA was successful in knocking down Kv3.3 channels. If results of IHC show that Kv3.3 was not sufficiently knocked down, Kv3.3-knockout mice may need to be used to examine the role of Kv3.3 in MSA response to stretch. Finally, other channels that have not yet been discovered in the muscle spindles may play a role in OX-induced neuropathy; further studies could use IHC to determine if previously undiscovered channels are present in the spindles, and if knocking down any of those channels significantly reproduces MSA firing patterns in OX-treated animals.

TABLES AND FIGURES

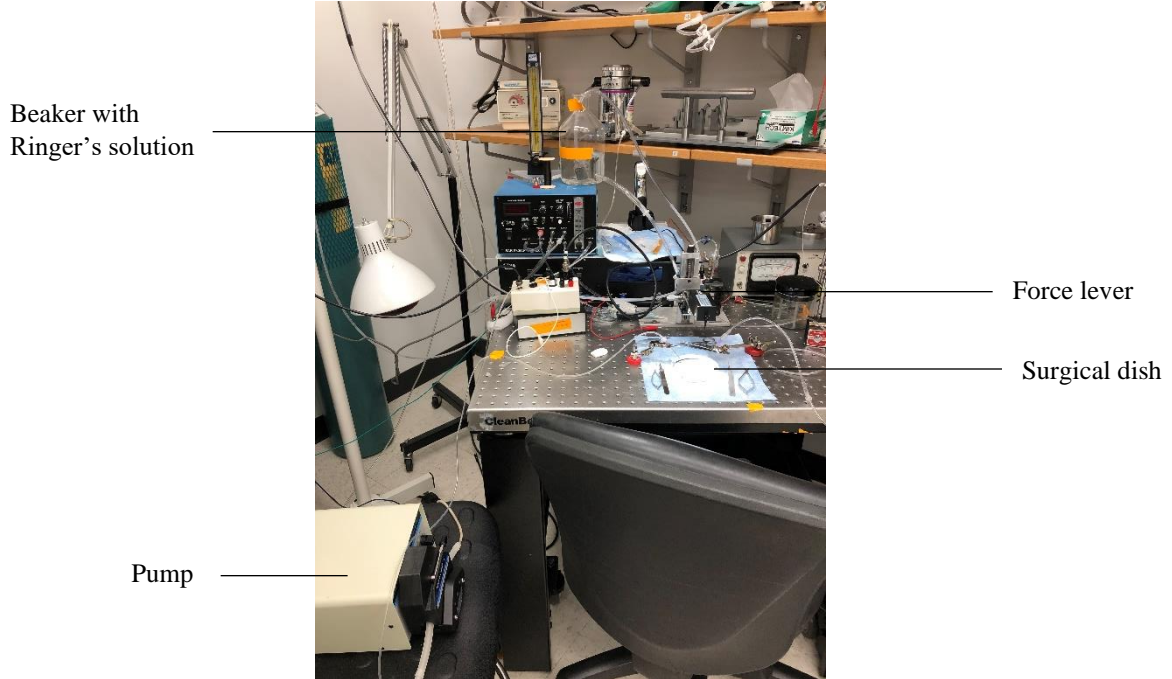


Figure 1. Experimental setup with beaker of Ringer's solution, surgical preparation area, and pump (bottom left).

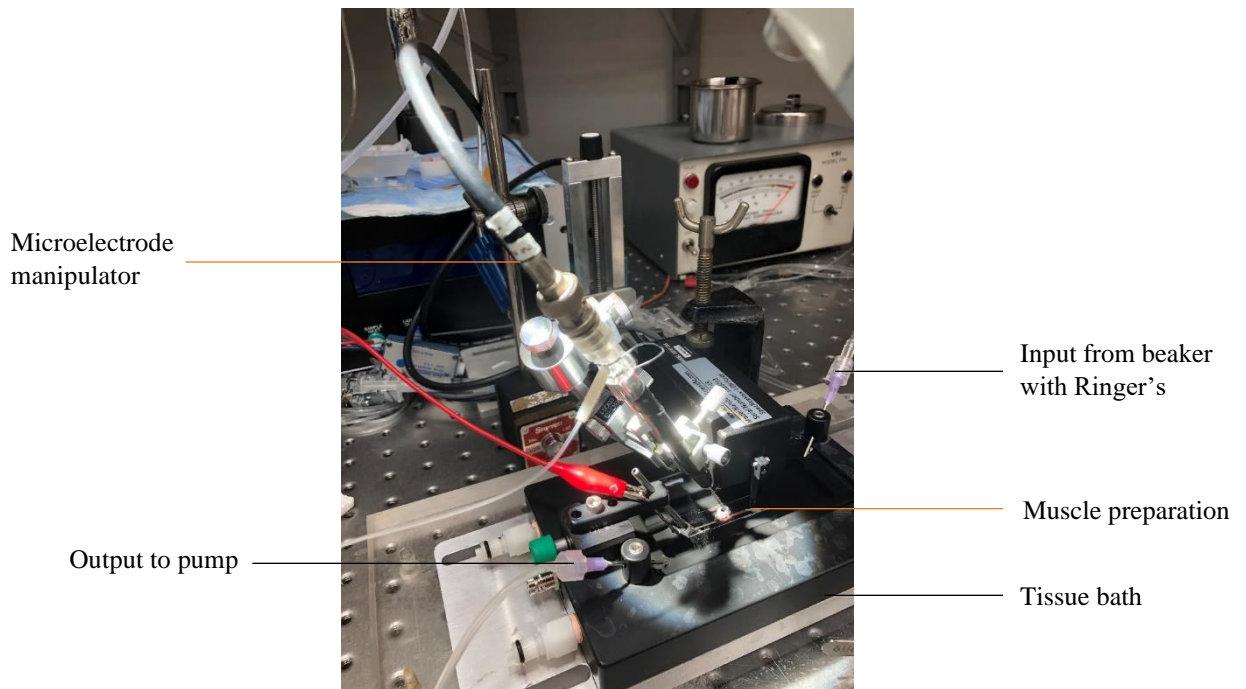


Figure 2. Apparatus for recording muscle afferents.

Type of Stretch	Amplitude (mm)	Velocity (mm/s)	Number Per Afferent
Ramp with Constant Velocity	0.5	20	4
	0.75	20	4
	1.00	20	4
	1.25	20	4
Three-Triangles			3
Ramp with Constant Amplitude	1.00	5	4
	1.00	10	4
	1.00	15	4
	1.00	20	4

Table 1. Amplitude and velocities of the stretches conducted for each afferent.

Type	Criteria		
	Pause in isometric twitch stretch	Perfect entrainment to high frequency vibrations	High instantaneous firing frequency at onset of dynamic stretch
Ia	Yes	Yes	Yes
Ib	No	-	-
II	Yes	No	No

Table 2. Classification of afferents based on responses to stretch.

REFERENCES

- [1] Siegel, R. L., Miller, K. D., Sauer, A. G., Fedewa, S. A., Butterly, L. F., Anderson, J. C., . . . Jemal, A. (2020). Colorectal cancer statistics, 2020. *CA: A Cancer Journal for Clinicians*, 70(3), 145-164.
- [2] Raymond, E., Faivre, S., Woynarowski, J. M., & Chaney, S. G. (1998, April). Oxaliplatin: mechanism of action and antineoplastic activity. In *Seminars in oncology* (Vol. 25, No. 2 Suppl 5, p. 4).
- [3] Vincent, J. A., Wiczerzak, K. B., Gabriel, H. M., Nardelli, P., Rich, M. M., & Cope, T. C. (2016). A novel path to chronic proprioceptive disability with oxaliplatin: distortion of sensory encoding. *Neurobiology of disease*, 95, 54-65.
- [4] Bullinger, K. L., Nardelli, P., Wang, Q., Rich, M. M., & Cope, T. C. (2011). Oxaliplatin neurotoxicity of sensory transduction in rat proprioceptors. *Journal of neurophysiology*, 106(2), 704-709.
- [5] Housley, S. N., Nardelli, P., Carrasco, D. I., Rotterman, T. M., Pfahl, E., Matyunina, L. V., . . . Cope, T. C. (2020). Cancer Exacerbates Chemotherapy-Induced Sensory Neuropathy. *Cancer Research*, 80(13), 2940-2955.
- [6] Kaczmarek, L. K., & Zhang, Y. (2017). Kv3 channels: enablers of rapid firing, neurotransmitter release, and neuronal endurance. *Physiological reviews*, 97(4), 1431-1468.
- [7] Dell'Orco, J. M., Pulst, S. M., & Shakkottai, V. G. (2017). Potassium channel dysfunction underlies Purkinje neuron spiking abnormalities in spinocerebellar ataxia type 2. *Human molecular genetics*, 26(20), 3935-3945.

- [8] Gebremedhn, E.G., Shortland, P.J. & Mahns, D.A. The incidence of acute oxaliplatin-induced neuropathy and its impact on treatment in the first cycle: a systematic review. *BMC Cancer* 18, 410 (2018).
- [9] Sisignano, M., Baron, R., Scholich, K., & Geisslinger, G. (2014). Mechanism-based treatment for chemotherapy-induced peripheral neuropathic pain. *Nature reviews. Neurology*, 10(12), 694–707.
- [10] André, T., Boni, C., Mounedji-Boudiaf, L., Navarro, M., Tabernero, J., Hickish, T., . . . Gramont, A. D. (2004). Oxaliplatin, Fluorouracil, and Leucovorin as Adjuvant Treatment for Colon Cancer. *New England Journal of Medicine*, 350(23), 2343-2351.
- [11] Saif, M. W., & Reardon, J. (2005). Management of oxaliplatin-induced peripheral neuropathy. *Therapeutics and clinical risk management*, 1(4), 249.
- [12] Joho, R. H., Street, C., Matsushita, S., & Knopfel, T. (2006). Behavioral motor dysfunction in Kv3-type potassium channel-deficient mice. *Genes, Brain and Behavior*, 5(6), 472-482.
- [13] Ellaway, P. H., Taylor, A., & Durbaba, R. (2015). Muscle spindle and fusimotor activity in locomotion. *Journal of anatomy*, 227(2), 157-166.
- [14] Bewick, G. S., & Banks, R. W. (2015). Mechanotransduction in the muscle spindle. *Pflügers Archiv-European Journal of Physiology*, 467(1), 175-190.
- [15] Vincent, J. A., Gabriel, H. M., Deardorff, A. S., Nardelli, P., Fyffe, R. E., Burkholder, T., & Cope, T. C. (2017). Muscle proprioceptors in adult rat: mechanosensory signaling and synapse distribution in spinal cord. *Journal of neurophysiology*, 118(5), 2687-2701.

- [16] Vincent, J. A., Wiczerzak, K. B., Gabriel, H. M., Nardelli, P., Rich, M. M., & Cope, T. C. (2016). A novel path to chronic proprioceptive disability with oxaliplatin: distortion of sensory encoding. *Neurobiology of disease*, 95, 54-65.
- [17] Hsieh, J. Y., Ulrich, B. N., Issa, F. A., Meng-chin, A. L., Brown, B., & Papazian, D. M. (2020). Infant and adult SCA13 mutations differentially affect Purkinje cell excitability, maturation, and viability in vivo. *Elife*, 9, e57358.
- [18] Dell'Orco, J. M., Pulst, S. M., & Shakkottai, V. G. (2017). Potassium channel dysfunction underlies Purkinje neuron spiking abnormalities in spinocerebellar ataxia type 2. *Human molecular genetics*, 26(20), 3935-3945.
- [19] Franco, J. A., Kloefkorn, H. E., Hochman, S., & Wilkinson, K. A. (2014). An in vitro adult mouse muscle-nerve preparation for studying the firing properties of muscle afferents. *JoVE (Journal of Visualized Experiments)*, (91), e51948.