Modeling of neuronal excitability in response to application of TMS and neurotoxins to abnormal neurons

Armen Gharibans, Marianne Catanho, Mridu Sinha
Department of Bioengineering
Jacobs School of Engineering
University of California, San Diego

Abstract

Chronic pain is estimated to afflict millions of people worldwide, affecting their well-being and quality of life. Due to the complexities of the human brain, it can often be very challenging to treat with only 40-60% of patients achieving partial relief. The source of chronic pain is neurons with abnormal and hyper-excitable firing properties. Botulinum toxin, similar to other neurotoxins, interacts with the neurotransmitter release vesicles and prevents the release of acetylcholine, which can be used to reduce the hyperactivity of pain neurons. Transcranial magnetic stimulation (TMS) is a noninvasive method of brain stimulation that can alter the firing frequency of neurons. This paper investigates the use of neurotoxin in conjunction with TMS in restoring normal neuronal behavior.

1 Introduction

Chronic pain is estimated to afflict millions of people worldwide, affecting their well-being and quality of life [1]. Due to the complexities of the human brain, it can often be very challenging to treat with only 40-60% of patients achieving partial relief [2].

Literature suggests the presence of selective synaptic connections and molecular signaling in pain related cortical areas. Chronic pain is caused by plastic changes or long term potentiation in cortical synapses. It is associated with lesions in the nervous system and often involves abnormal reinnervation and neuronal sprouting after trauma [3]. Neurons involved in these lesions present abnormal, hyper-excitable firing properties regardless of any stimulus. This could be caused by either a reduction in pain threshold (Allodynia) or an enhanced response to noxious stimuli (Hyperalgesia). Chronic pain persists after an injury has healed and results from significant functional and structural changes in the nervous system. Hence, chronic pain has been proposed to be the persistence of and/or inability to extinguish the memory of pain evoked by an initial inciting injury [4]. Even though the exact mechanism remains unknown, the anterior cingulate cortex (ACC) has been found of play an important role. The ACC responds to persistent nociceptive stimulation with significant plasticity, which contributes to the maintenance of chronic pain.

Transcranial magnetic stimulation (TMS) is a noninvasive method of brain stimulation, which uses electromagnetic induction to induce weak electric currents. TMS causes neurons in the neocortex under the site of stimulation to depolarize and discharge an action potential. A train of pulses can result in either inhibition or facilitation depending on the frequency. TMS activates the thalamic nuclei connected to the motor and premotor cortex, which causes a cascade of events in pain related structures receiving inputs from these nuclei, including medial thalamus, ACC, and upper brainstem (Figure 1)[5]. TMS is a promising, non-invasive treatment option for chronic neuropathic pain that can be used to alter the firing frequency of neurons. However, most literature indicates that TMS has little therapeutic value, often confused with placebo effects. We suspect this is a result of non-

physiological frequencies used during stimulation, since literature also shows that the response of neurons to external stimuli is dependent upon their intrinsic resonant frequencies [6].

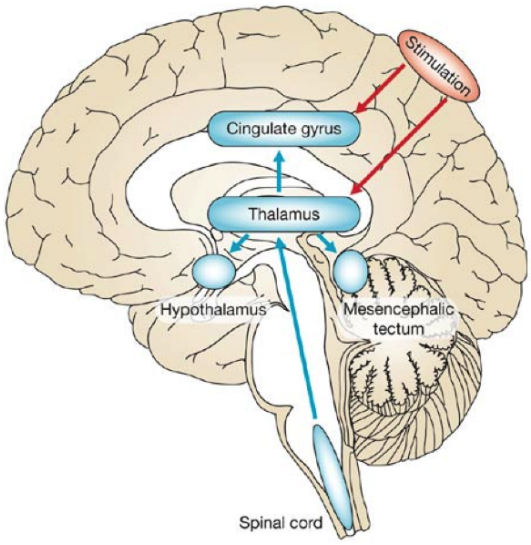

Figure 1: Cascade effect during TMS [5].

Botulinum toxin, similar to other neurotoxins, interacts with the neurotransmitter release vesicles and prevents the release of acetylcholine. Botulinum cleaves the proteins associated with vesicle fusion into the plasma membrane, leading to the inhibition of neurotransmission. Specifically, cleaving of those proteins creates a nonfunctional vesicle fusion complex impacting Ca^{2+} influx and fusion [7].

In this project, we propose a model for treatment of chronic pain. We investigate the use of TMS stimulation, in conjunction with Botulinum toxin to restore the intrinsic firing pattern of the pain neurons. The botulinum was explored as a means to reduce the hyper excitability of pain neurons, while TMS was used to retrain the pain neurons to fire at their intrinsic resonant frequency.

2 Methods

2.1 Compartmental model: modeling normal cortical neurons and abnormal neurons

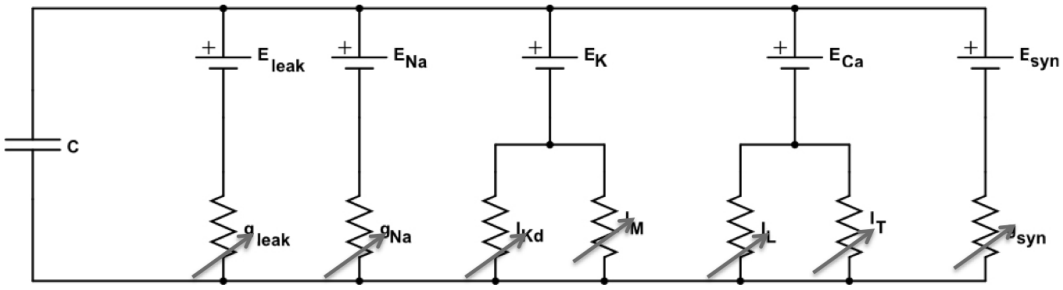

Figure 2: Compartmental model of cortical neurons used in the project.

Intrinsic electrophysiological properties were modeled using a common Hodgkin-Huxley (HH) type model for cortical neurons for regular spiking class cells (Figure 2). The equations for the single-compartment neuronal model were selected from literature [8] to account for spike-frequency adaptation and presence or absence of burst discharges from depolarizing stimuli or following hyper polarizing inputs.

Cortical neurons categorized as abnormal (or "pain" neurons) were modeled as injured neurons. Following injury, literature suggests that neurons display change in excitability due to increased sodium channel expression [9,10]. An increase in K^+ channels' conductance has been linked anti-nociceptive effects in models of chronic pain [9]. In the proposed model, regular firing patterns are modified through increases in Na^{2+} conductance and decreases in K^+ conductance for aberrant neurons.

Computational models were run in MATLAB simulation environment. Only single-compartment neurons were modeled, and are described by the following equations.

2.1.1 Membrane Equation

$$C_m \frac{dV}{dt} = -g_{leak}(V - E_{leak}) - I_{Na} - I_K - I_M - I_T - I_L \tag{1}$$

where V is the membrane voltage, C_m = 0.29 $\mu F/cm^2$ is the membrane capacitance, g_{leak} and E_{leak} are the resting membrane conductance and reversal potential.

We utilized five ionic current models: sodium current (I_{Na}), potassium current (I_K), slow-inactivating voltage-dependent potassium current (I_M) (spike-frequency adaptation), high-threshold calcium current (I_L) and low-threshold calcium current (I_T).

The details for each HH voltage-dependent current are given below. The choice of currents was arbitrary and driven by the need of certain channels to represent pain and neurotoxin effect. Conductance, voltage and other variable values were extracted from literature on HH models of cortical neurons.

2.1.2 Sodium voltage-dependent current

This sodium current was modified from HH models and the below equations have been particularly used in models of cortical pyramidal cells.

$$I_{Na} = g_{Na} m^3 h (V - E_{Na}) \tag{2}$$

$$\frac{dm}{dt} = \alpha_m(V)(1 - m) - \beta_m(V)m \tag{3}$$

$$\frac{dh}{dt} = \alpha_h(V)(1 - h) - \beta_h(V)h \tag{4}$$

$$\alpha_m = \frac{-0.32(V - V_T - 13)}{exp(-(V - V_T - 13)/4) - 1} \tag{5}$$

$$\beta_m = \frac{0.28(V - V_T - 40)}{exp(-(V - V_T - 40)/5) - 1} \tag{6}$$

$$\alpha_h = 0.128 exp(-(V - V_T - 17)/18) \tag{7}$$

$$\beta_h = \frac{4}{1 + exp(-(V - V_T - 40)/5)} \tag{8}$$

where g_{Na} = 56 mS/cm^2, E_{Na}=50mV and V_T = =-56.16mV, adjusts spike threshold.

2.1.3 Potassium voltage-dependent current

We model the delayed-rectifier K^+ current:

$$I_K = g_K n^4 (V - E_K) \tag{9}$$

$$\frac{dn}{dt} = \alpha_n(V)(1 - n) - \beta_n(V)n \tag{10}$$

$$\alpha_n = \frac{-0.032(V - V_T - 15)}{exp(-(V - V_T - 15)/5) - 1} \tag{11}$$

$$\beta_n = 0.5 exp(-(V - V_T - 10)/40) \tag{12}$$

where where g_K = 6 mS/cm^2 and E_K=-90mV.

2.1.4 Slow-inactivating potassium current

The slow-inactivating potassium current (I_M) is responsible for spike-frequency adaptation, and was modeled as:

$$I_M = g_M p(V - E_K) \tag{13}$$

$$\frac{dp}{dt} = (p_\infty(V) - p)/\tau_p(V) \tag{14}$$

$$p_\infty = \frac{1}{1 + exp(-(V + 35)/10)} \tag{15}$$

$$p_\infty = \frac{\tau_{max}}{3.3exp((V + 35)/20) + exp(-(V + 35)/20)} \tag{16}$$

where where g_M = 0.075 mS/cm^2 and τ_{max}=4s.

2.1.5 High-threshold calcium current

The first type of Ca^{2+} was modeled with the following equations:

$$I_L = g_L q^2 r(V - E_{Ca}) \tag{17}$$

$$\frac{dq}{dt} = \alpha_q(V)(1 - q) - \beta_q(V)q \tag{18}$$

$$\frac{dr}{dt} = \alpha_r(V)(1 - r) - \beta_r(V)r \tag{19}$$

$$\alpha_q = \frac{0.055(-27 - V)}{exp((-27 - V)/3.8) - 1} \tag{20}$$

$$\alpha_r = 0.000457exp((-13 - V)/50) \tag{21}$$

$$\beta_q = 0.94exp((-75 - V)/17) \tag{22}$$

$$\beta_r = \frac{0.0065}{exp((-15 - V)/28) + 1} \tag{23}$$

where g_L = 0.22 mS/cm^2 and E_{Ca} = 120mV.

2.1.6 Low-threshold calcium current

The low-threshold calcium current, I_T, responsible for rebound bursts, was modeled as follows:

$$I_T = g_T s_\infty^2 u(V - E_{Ca}) \tag{24}$$

$$\frac{du}{dt} = (u_\infty(V) - u)/\tau_u(V) \tag{25}$$

$$s_\infty(V) = \frac{1}{1 + exp(-(V + V_x + 57)/6.2)} \tag{26}$$

$$u_\infty(V) = \frac{1}{1 + exp(-(V + V_x + 81)/4)} \tag{27}$$

$$t_u(V) = \frac{30.8 + (211.4 + exp((V + V_x + 113.2)/5))}{3.7(1 + exp((V + V_x + 84)/3.2)} \tag{28}$$

where g_T = 0.35 mS/cm^2 and V_x = 2mV.

2.2 Cascade model

We considered two scenarios in order to study the effect of TMS and neurotoxin:

1. TMS and neurotoxin acting on a single cortical pain neuron, modeled with the same set of equations and constants as described above, with changes in the K^+ and Ca^{2+} conductances [9, 10].

2. A cascade of neurons, synaptically connected, with a gradient of TMS applied to cortical neurons in the ACC and the neurotoxin locally delivered around the upper brain stem region (Figure 3). For this scenario, we considered three normal neurons distributed across ACC and two abnormal (pain) neurons in the brain stem with those neurons under indirect TMS effect due to the synaptic connections.

While the first scenario can be used to study the effect of TMS and neurotoxin on the pain neuron, given the non specific nature of the magnetic field induced by TMS, it is not feasible to specifically target the pain neurons. Also, non-superficial cortex areas are often associated with pain sensing and processing. Therefore, for the purposes of this project, we assume that the neurotoxin can be delivered locally to the pain neurons' area.

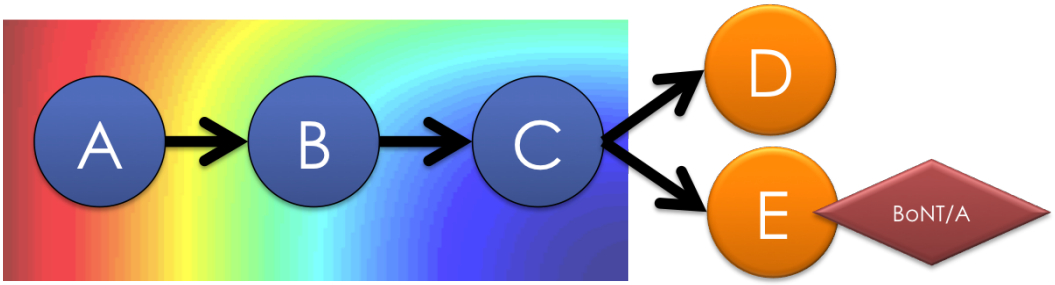

Figure 3: Cascade effect. A, B and C are neurons in the ACC, D and E are neurons in the brain stem. Only E receives neurotoxin.

2.3 TMS Model

First, we modeled the pulsating electromagnetic field generated by the TMS coil placed above the skull over the motor cortex. Then we modeled the ionic current induced by the electric field along the length of the neuron.

2.3.1 Modeling Electromagnetic Field

Literature suggests that neurons are insensitive to the transverse field simulation relative to axial field simulation [11]. Hence, we only considered the components of electric field in the plane of the motor cortex (E_x, E_y in Figure 4) and neglected the field perpendicular to the neuronal membrane. In our model, we assumed that the length of all the target neurons lies in the same plane, and disregarded any bent neurons.

We considered a magnetic field characterized by a square wave with 10 % duty cycle with a frequency of 40Hz. This frequency corresponded to the intrinsic resonant frequency of the cortical neurons under consideration. The electric field was modeled over a 500μm x 500μm area on the motor cortex with a resolution of 1μm:

$$\vec{E} = -\frac{\partial I}{\partial t}\frac{\mu N}{\pi k}\left(\frac{r}{x}\right)^{1/2}\left[K(m)\left(1-(\frac{1}{2})k^2\right)-E(m)\right]\hat{\theta} \tag{29}$$

2.3.2 Modeling induced current

The magnetic field generated by the TMS coil induces an electric field in the brain which results in a current. In our model, the current was added to the neuronal model as external current to simulate

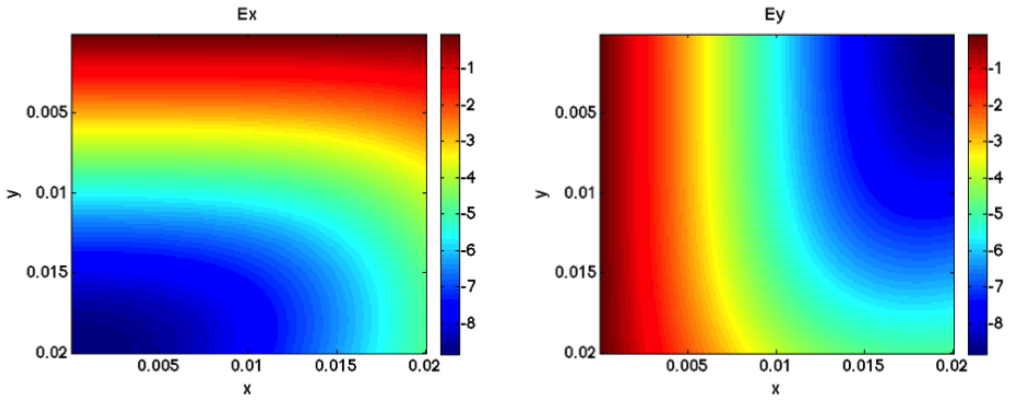

Figure 4: Electromagnetic field in the plane of the motor cortex.

the effect of TMS. The length of neurons were assumed to be 96μm. The position of the neuron and its orientation was randomly selected to calculate the current induced by the electric field along the length of the neuron [12].

For analyzing the effect of TMS on a single neuron, the neuron was assumed to be at a distance of 2cm from the coil in the ACC. In order to study the cascade effect (Figure 3), the three neurons at the start (A), center (B) and end (Cs) of ACC were selected. We assumed that the ACC is 2cm away from the coil and with a thickness of 1.8cm. Therefore, we modeled three neurons at a distance of 2cm, 2.7cm and 3.8cm from the coil.

The induced current and electric field are given by:

$$\tilde{I}_a = \frac{\vec{E} \cdot \hat{a}}{r_a} \tag{30}$$

$$\vec{E} = E_x \hat{x} + E_y \hat{y} \tag{31}$$

$$\vec{E} \cdot \hat{a} = E_x \frac{(x_1 - x_0)}{a} + E_y \frac{y_1 - y_0}{a} \tag{32}$$

$$\tilde{i}_m = -\frac{1}{a r_a} \{[E_x(x_1, y_1) - E_x(x_0, y_0)](x_1 - x_0) + [E_y(x_1, y_1) - E_y(x_0, y_0)](y_1 - y_0)\} \tag{33}$$

We explored different parameters sets (final adopted values are shown in parenthesis) for coil diameter (0.04cm), number of coils (100), frequency (40Hz) and duty cycle (10 %) to get a physiologically meaningful value of induced current [12].

2.4 Effect of neurotoxin

From literature, we know that neurotoxins directly affect signaling processes [13]. Botulinum (BoNT/A) is known to have an effect in Ca^{2+} conductance through inhibition of vesicular release of acetylcholine neurotransmitter [13]. Thus, we assumed that injection of Botulinum decreases Ca^{2+} conductance in the targeted cells. We used the Kuba and Nishi's synaptic input equation below, to model the change in calcium conductance due to neurotoxin.

$$g_B(t) = [BoNT/A]\, t e^{-t/t_peak}$$

where the concentration of neurotoxin is given by $[BoNT/A] = 0.300mM$ and t_{peak}, where peak conductance is achieved, was chosen arbitrarily (300ms). The effect of neurotoxin was only applied to the neurons in the model that are in the brain stem.

3 Results

3.1 Normal neuronal firing vs abnormal (pain) firing

The normal neuron fires at the expected frequency of 40Hz, while the pain neurons show abnormal bursts of neuronal firing (obtained through an changes in K^+ and Ca^{2+} conductances) (Figure 5). No TMS or neurotoxin are present at this point in the simulation.

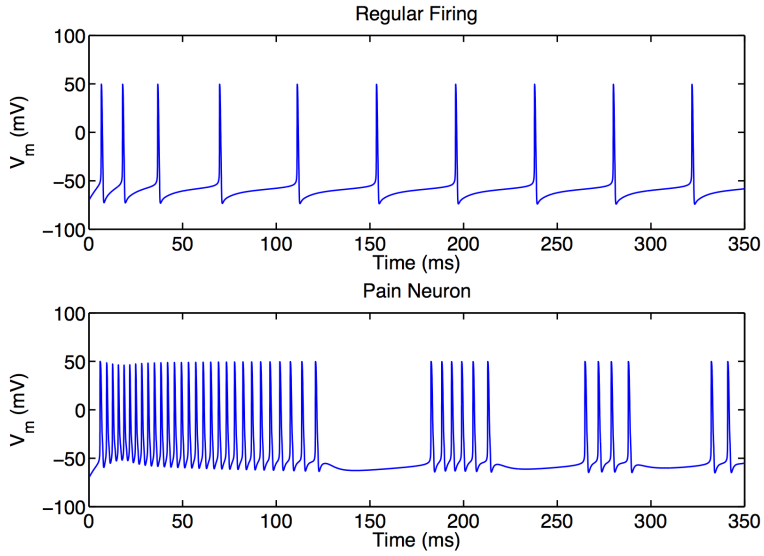

Figure 5: Membrane voltage for normal cortical neuron (top) and aberrant neuronal firing pattern (bottom).

3.2 Single neuron - effect of TMS, neurotoxin, and TMS+neurotoxin concurrently applied

Our results show that the TMS alone fails to regulate the firing pattern of the pain neuron to back to normal state (Figure 6). Neurotoxin on the other hand removes the abnormal bursting pattern of the pain neurons, but it is unable to bring the frequency of the neuronal firing to the expected normal value.

However, when both neurotoxin and TMS are applied simultaneously to the pain neuron, we are able to train the pain neuron to fire at its intrinsic resonant frequency. This supports our hypothesis that neurotoxin can be applied to reduce the hyperactivity of pain neurons. TMS can then be used to train them to fire at a certain frequency. Over time, we expect this method to induce plastic changes to the pain neuron if the frequency of TMS is in line with the intrinsic resonant frequency of the neuron.

3.3 Cascade effect

We simulated the proposed cascade effect (Figure 3) by connecting neurons A, B, C (ACC neurons), D and E with standard excitatory synapses. D (pain neuron, no neurotoxin) and E (pain neuron, neurotoxin) only receive input from neuron C. The result of the cascade effect (indirect TMS) on D and E is shown in Figure 7.

The results here support our findings in with the single neuron simulation. Even though TMS can not be use to specifically target the pain neuron, the effect of TMS can be trickled down to the pain neuron through a cascade of synaptic connections between the normal neurons in the ACC that receive TMS and the pain neuron. Results show that the pain neuron, with synaptic connections from normal neurons receiving the TMS, can be trained to fire at a determined frequency under the effect of neurotoxin.

4 Conclusion

We modeled the effect of neurotoxins and TMS directly in the cortical neurons. We also modeled the effect of TMS on the pain neuron through a cascade of synaptic connections. Our hypothesis was that neurotoxins, such as Botulinum, can be applied to reduce the hyperactivity of pain neurons and TMS could then be used to train them to fire at certain frequency.

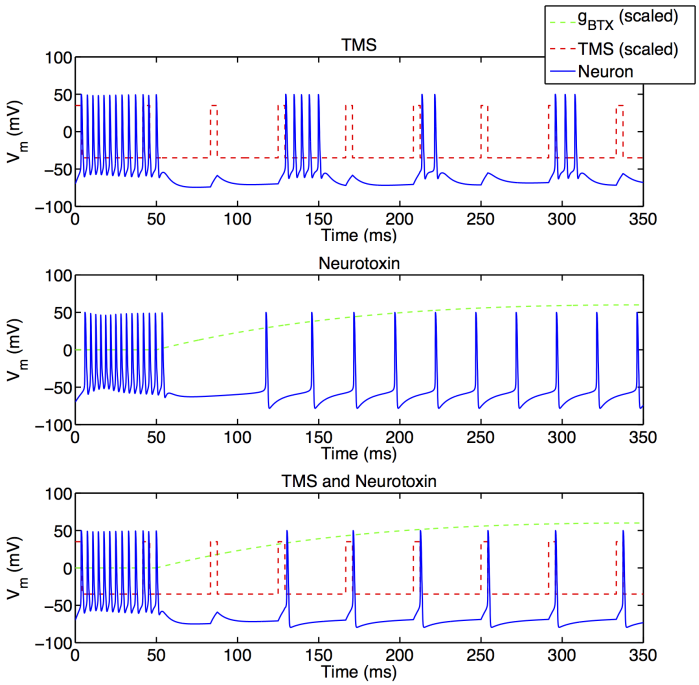

Figure 6: Membrane voltage of cortical pain neuron in the presence of TMS (top), neurotoxin (middle) and TMS in conjunction with neurotoxin (bottom). Neurotoxin-induced change of conductance starts at t=50ms.

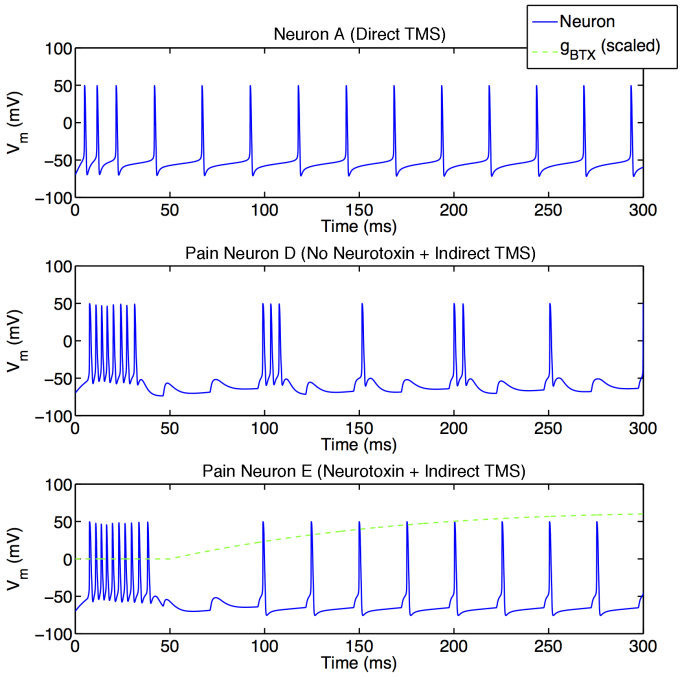

Figure 7: Membrane voltage of normal cortical neuron A in the presence of TMS (top), of pain neuron D (middle) in the absence of neurotoxin but with an excitatory synaptic input from normal neuron C, and of pain neuron (bottom) E in the presence of neurotoxin and excitatory synaptic (indirect TMS) input from normal neuron C. Neurotoxin-induced change of conductance starts at t=50ms.

Our results show that the TMS alone, applied either directly to the pain neurons or trickled-down through synaptic connections from normal neurons, fails to regulate the firing pattern of the pain neuron to a normal firing. Neurotoxin reduces firing frequencies, as expected, but does not restore the neuron's intrinsic firing patterns. However, when neurotoxin and TMS are applied simultaneously to the pain neuron (through either direct application or synaptic cascade effect) we are able to train the pain neuron to fire at some determined frequency. We expect that, if stimulation is given at the neuron's intrinsic resonant frequency, eventually plastic changes would drive the pain neuron to its normal state.

This model provides a good starting point for future studies. In addition, more detail research needs to be performed to account for the brain's complex structure. In-vivo experiments are also needed to validate these findings. Nevertheless, combining TMS and neurotoxins such as Botulinum show promise in the treatment of chronic pain due to abnormal neuronal firing.

References

[1] N Becker, et al. (1997). "Pain epidemiology and health related quality of life in chronic non-malignant pain patients referred to a Danish multi-disciplinary pain center". Pain. 73, pp. 393-400.

[2] Dworkin RH, et al. (2007). "Pharmacologic management of neuropathic pain: evidence-based recommendations". Pain. 132, pp. 237-51.

[3] Costigan, et al. (2009). "Neuropathic pain: a maladaptive response of the nervous system to damage." Annual Review of Neuroscience". 32: 1.

[4] Apkarian AV, et al. (2009). "Towards a theory of chronic pain." Prog Neurobiol. 87, pp. 81-97.

[5] F. Fregni, A.P. Pascual-Leone (2007) "Technology insight: noninvasive brain stimulation in neurology-perspectives on the therapeutic potential of rTMS and tDCS." Nat Clin Pract Neurol, 3, pp. 383-393

[6] Salansky N, et al. (1998). "Responses of the nervous system to low frequency stimulation and EEG rhythms: clinical implications." NeurosciBiobehav Rev. 22, pp. 395-409.

[7] Dickerson JT, et al. (2006). "The Use of Small Molecules to Investigate Molecular Mechanism and Therapeutic Targets for Treatment of Botulinum Neurotoxin A Intoxication". ACS Chem Biol. 1, pp. 359-359.

[8] Pospischil M, et al. (2008). "Minimal Hodgkin-Huxley type models for different classes of cortical and thalamic neurons." Biological Cybernetics 99.4, pp. 427-441.

[9] Ocana M, et al. (2004). "Potassium channels and pain: present realities and future opportunities." European journal of pharmacology 500.1-3, p. 203.

[10] Waxman SG, et al. (1999) "Sodium channels and pain." Proceedings of the National Academy of Sciences 96.14, pp. 7635-7639.

[11] Pashut T, et al. (2011) "Mechanisms of magnetic stimulation of central nervous system neurons." PLoS Comput. Biol., 7, pp. 1002-1022.

[12] Kobayashi and Pascual-Leone (2003) "Transcranial magnetic stimulation in neurology." Lancet Neurol, 2, pp. 145-156.

[13] Kuba K., and Nishi S. (1979) "Characteristics of fast excitatory postsynaptic current in bullfrog sympathetic ganglion cells." Pflugers Archiv European Journal of Physiology 378.3, pp. 205-212.