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Serotonergic Modulation of Motoneuronal Excitability and Its Impact on Muscle Force Generation: A Computational Study

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Abstract

This study investigated the modulatory effects of serotonin (5-HT) on motor neuron excitability and muscle force generation using a computational model. The impact of 5-HT receptor activation was evaluated across a range of stimulation frequencies. Physiological concentrations of 5-HT significantly enhanced motor neuron excitability at 40 Hz and 100 Hz ($p < 0.0001$), consistent with the excitatory role of 5-HT$_2$A receptor activation. In contrast, supraphysiological levels of 5-HT reduced excitability, likely due to overactivation of inhibitory 5-HT$_1$A receptors, which induce neuronal hyperpolarization. Muscle force output was positively correlated with motor neuron activity. At 10 Hz, physiological 5-HT release had no significant effect on force generation, suggesting minimal influence at low stimulation frequencies. However, elevated 5-HT levels shortened contraction duration, indicating the onset of muscle fatigue ($p < 0.0001$). At higher frequencies, physiological 5-HT supported sustained muscle contractions, whereas excessive 5-HT impaired contraction maintenance, promoting fatigue ($p < 0.0001$). These results are consistent with experimental observations and support the validity of the model as a reliable tool for investigating serotonergic modulation of neuromuscular function. The model provides mechanistic insights into fatigue development and may inform therapeutic strategies targeting serotonergic pathways to improve muscle performance and recovery. Future studies should explore interactions with other neurotransmitter systems to further elucidate their contributions to motor control and fatigue resistance.

Keywords: Serotonin; Motor neurons; Muscle contraction; Neuromuscular fatigue; Computational modeling.

Introduction

The function of skeletal muscles is determined by the activation of motor units (MUs), which are modulated by motoneurons in response to synaptic signals. Neurotransmitters play a key role in motor neuron excitability and, consequently, in muscle force production [1]. Among these neurotransmitters, serotonin (5-HT) is particularly relevant because its action on different receptors can modulate muscle fatigue and motor control efficiency [2]. However, the interaction between 5-HT1a and 5-HT2a receptors on neuronal excitability and their impact on force production remain poorly understood. 5-HT1a and 5-HT2a receptors have been shown to affect motoneuron excitability [3] differentially. While 5-HT1a receptor inhibits neuronal activity and contributes to fatigue, 5-HT2a receptor promotes sustained motoneuron activation, suggesting an adaptive mechanism to maintain muscular effort without a rapid decline in performance. Nevertheless, an imbalance in the activation of these receptors can be detrimental, as elevated serotonin levels have been linked to central fatigue [4]. During muscle activity, neurons in the raphe nucleus release 5-HT onto the dendrites and soma of motoneurons, where excitatory 5-HT2a receptors are abundant. However, if neurotransmitter release is excessively high, inhibitory 5-

HT1a receptors are also activated. These receptors are located in the initial segment of the axon and modulate action potential propagation by inhibiting Na^+ channels [5].

To better understand the role of serotonin in muscle fatigue modulation, it is crucial to develop computational models that accurately replicate the interaction between serotonergic receptors and motor neurons. This study presents a model using Python's NEURON module to simulate motoneuron activation in response to serotonin release from presynaptic neurons. The motoneuron is further connected to a muscle model, enabling the evaluation of force production as a function of the excitability modulated by 5-HT. This approach allows for the exploration of how the differential activation of 5-HT1a and 5-HT2a receptors influences muscle contraction dynamics and fatigue generation.

Materials and Methods

Technical Information

The model consists of a motoneuron and generic supraspinal presynaptic neuron (raphe nuclei) that releases the neurotransmitter 5-HT upon stimulation [5]. The modeling was developed with the Neuron 8.2 module of Python 3.12, in the Spyder development environment. This module is a simulation environment for modeling individual and network neurons, and provides a range of conventional tools for constructing, managing, and using modeling in a numerically robust and computationally efficient manner [6]. The script files of the model developed in this study are publicly available at https://github.com/JGMG7/Serotonergic-Modulation-of-Motoneuronal-Excitability-and-Muscle-Force-Generation-Computational-Study.

Motoneuron

The motoneuron models used in this study were based on Kim [7], Fietkiewicz et al. [6], and Meléndez-Gallardo [8]. This model consists of a multi-compartmental cable model with anatomical data corresponding to a cat motoneuron (i.e., v_e_moto6), which is available in the public database (www.neuromorph.org). The non-uniform specific membrane resistivity was assigned to soma (Rm, soma = 225 $\Omega \cdot cm^2$) and dendrites (Rm, dendrite = 225 $\Omega \cdot cm^2$). Specific membrane capacitance (Cm = 1 $\mu F/cm^2$) and axial resistivity (Ri = 70 $\Omega \cdot cm$) were uniformly assigned to all compartments of the motoneuron model. Action potentials and afterhyperpolarization phenomena were generated by various Hodgkin-Huxley-type active currents: fast-inactivating sodium current (I_Naf), delayed rectifier potassium current (I_KDr), persistent sodium current (I_Nap), N-type calcium current (I_CaN), and calcium-dependent potassium current [I_K(Ca)] in the soma. In addition, I_Naf, I_Nap, and I_KDr were included in the initial segment/axon hillocks. The peak conductances for active currents were G_Naf = 0.71 S/cm^2, G_KDr = 0.23 S/cm^2, G_CaN = 0.013 S/cm^2, and G_K(Ca) = 0.0258 S/cm^2 at the soma, while at the initial segment/axon hillock, they were G_Naf = 2.7 S/cm^2, G_Nap = 0.033 mS/cm^2, and G_KDr = 0.17 S/cm^2. To generate the inhibitory and excitatory responses of the motoneuron, 5-HT receptors of subtypes 5-HT1a and 5-HT2a are incorporated into the initial segment, dendrites, and soma, respectively [3].

Muscle Unit

The muscle unit consists of a section that incorporates two mechanisms: calcium and force. The force mechanism relies on variables derived from the calcium mechanism for activation. Additionally, the muscle communicates with the motoneuron spike timing through a NetCon connection, utilizing pointers to ensure smooth interaction between biomechanically distinct elements. The equations governing these processes are detailed by Fietkiewicz et al. [6].

Presynaptic Neuron

The presynaptic neuron is modeled as a generic neuron (raphe nuclei), whose primary function is to release neurotransmitters upon receiving a stimulus (IClamp). It contains voltage-dependent Na^+ channels (with three states) and K^+ channels (with two states) following a Markov model [8,9].

It is assumed that upon the arrival of an action potential, the presynaptic terminal depolarizes, allowing Ca^{++} ions to enter and generating a high-threshold Ca^{++} current. These calcium ions then activate a calcium-binding protein that facilitates the release of serotonin (5-HT) from presynaptic vesicles into the synaptic cleft. Presynaptic vesicles are considered inexhaustible and always available for release. This process is modeled as a first-order reaction with a stoichiometric coefficient of n.

The entry of calcium into the presynaptic terminal is governed by a high-threshold Ca^{++} current using the same two-state Markov scheme as the K^+ channel, with a voltage-dependent rate similar to that of the K^+ current. Intracellular Ca^{++} removal is facilitated by active transport via a calcium pump. The 5-HT release mechanism interacts with 5-HT1a and 5-HT2a receptors on the motoneuron through the C pointer, which is in turn regulated by variables present in the rel2 mechanism (such as vesicle concentration, neurotransmitter molecules per vesicle, neurotransmitter hydrolysis rate, etc.) [8,9].

5-HT receptors

5-HT1a and 5-HT2a receptors were designed based on the kinetic response of G protein-coupled membrane receptors, which modulate ion channels [8,9]. The activity of both receptors is regulated by variables from the rel2 release mechanism of the presynaptic neuron via the C pointer. Specifically, for the 5-HT1a receptor, variations in the C pointer influence Ca^{++} currents in the motoneuron, while for the 5-HT2a receptor, fluctuations in the C pointer modulate Na+ and K^+ currents. The association (ka) and dissociation (kd) constants used to model 5-HT receptors were calculated as the average of the inhibition constant (ki) values reported in the literature [10]. Additionally, each receptor model has the ability to inhibit 5-HT reuptake, leading to increased neurotransmitter availability in the extracellular space. This mechanism is based on modifications to the variable kh, which acts as a constant that regulates the 5-HT hydrolysis rate, modeled as a first-order reaction [8,9]. The 5-HT2a receptor was inserted into the soma and the 311 dendrites of the motoneuron, whereas the 5-HT1a receptor was located in the initial segment.

Stimulation Protocols

The stimulation protocol consisted of NetStim generating simultaneous input to both the soma and dendrites (311) of the motoneuron, as well as to the raphe nucleus neuron (presynaptic neuron). The synaptic characteristics were set as weight = 2, tau = 0.5, and delay = 0, while the number of stimuli and interstimulus interval could be adjusted based on the required stimulus intensity.

Neurotransmitter Release

To simulate 5-HT release, we manipulated the nt variable in the rel2 neurotransmitter-release mechanism. The nt variable defines the number of molecules released each time a presynaptic neuron (raphe nucleus neuron) is excited. For what we defined as physiological release, the variable was set to nt = 10,000 5-HT molecules. For high-concentration neurotransmitter release, the variable nt was set to 1,000,000.

Statistical Analysis

The collected data were exported to*.csv format, and analyzed using the SciPy Python library [11] within the Spyder virtual environment. The Kruskal-Wallis test was applied, followed by Dunn's multiple comparison test. Significance threshold: $p > 0.05$, significant; ns = not significant.

Results

The motoneuron responses and muscle force generation were evaluated at three different stimulation frequencies (10, 40, and 100 Hz) under three distinct conditions, represented in the figures as a, b, and c: (a) no 5-HT receptor activity, (b) physiological 5-HT release, and (c) high-concentration 5-HT release. The muscle force recordings are also presented in the figures as d, e, and f, corresponding to conditions a, b, and c, respectively.

Motoneuron Electrical Activity and Muscle Force Generation at 10 Hz Stimulation

Motoneuron electrical activity varied significantly depending on the presence of 5-HT receptors and intensity of serotonergic discharge. At a stimulation frequency of 10 Hz, no significant differences were observed between the motoneuron activity without 5-HT receptors (condition a) and that with 5-HT1a and 5-HT2a receptors under physiological 5-HT release (condition b) ($p > 0.05$). However, a high 5-HT discharge (condition c) resulted in a significant decrease in electrical activity compared with both conditions a and b ($p < 0.0001$ in both cases) (Table 1, Figure 1).

Table 1. Statistical Analysis of Motoneuron Electrical Activity (Vm) Under Different Stimulation Frequencies

	Difference in rank sum	**p**	**Summary**
Stimuli 10 Hz			
Motoneuron (a) vs Motoneuron (b)	505.2	> 0.05	ns
Motoneuron (a) vs Motoneuron (c)	55860	< 0.0001	***
Motoneuron (b) vs Motoneuron (c)	55350	< 0.0001	***
Stimuli 40 Hz			
Motoneuron (a) vs Motoneuron (b)	1661	< 0.0001	***
Motoneuron (a) vs Motoneuron (c)	53690	< 0.0001	***
Motoneuron (b) vs Motoneuron (c)	52020	< 0.0001	***
Stimuli 100 Hz			
Motoneuron (a) vs Motoneuron (b)	1966	< 0.0001	***
Motoneuron (a) vs Motoneuron (c)	40990	< 0.0001	***
Motoneuron (b) vs Motoneuron (c)	39020	< 0.0001	***

Kruskal-Wallis Test and Dunn's Multiple Comparison Test. $p < 0.05$ = significant, ns = not significant

In terms of muscle force, at 10 Hz, no significant differences were observed between conditions (a) and (b) ($p > 0.05$), indicating that physiological 5-HT release did not significantly alter muscle force generation at low stimulation frequencies. However, high-concentration 5-HT release (c) led to a reduction in the force curve duration, suggesting the induction of fatigue ($p < 0.0001$) (Table 2, Figure 1).

Table 2. Statistical Analysis of Muscle Force (N) Generated Under Different Stimulation Frequencies.

	Difference in rank sum	**p**	**Summary**
Stimuli 10 Hz			
Muscle Force (a) vs Muscle Force (b)	0.0000	>0.05	ns
Muscle Force (a) vs Muscle Force (c)	18530	<0.0001	***
Muscle Force (b) vs Muscle Force (c)	18530	<0.0001	***
Stimuli 40 Hz			
Muscle Force (a) vs Muscle Force (b)	0,0000	>0.05	ns
Muscle Force (a) vs Muscle Force (c)	36850	<0.0001	***
Muscle Force (b) vs Muscle Force (c)	36850	<0.0001	***
Stimuli 100 Hz			
Muscle Force (a) vs Muscle Force (b)	0,0000	>0.05	ns
Muscle Force (a) vs Muscle Force (c)	49200	<0.0001	***
Muscle Force (b) vs Muscle Force (c)	49200	<0.0001	***

Kruskal-Wallis Test and Dunn's Multiple Comparison Test. $p < 0.05$ = significant, ns = not significant

Motoneuron Electrical Activity and Muscle Force Generation at 40 Hz Stimulation

At 40 Hz, significant differences were observed across all comparisons ($p < 0.0001$). The motoneurons with 5-HT receptors under physiological release (b) exhibited increased excitability compared to the no-receptor condition (a), whereas high 5-HT release (c) led to a decrease in excitability compared to both conditions (Table 1, Figure 2). Regarding the force curve, a shorter duration was observed in condition (b) than in condition (a), although the difference was not statistically significant ($p > 0.05$). In contrast, the force curve in condition (c) was significantly shorter than that in conditions(a) and (b) ($p < 0.0001$). The force profile showed sustained activation for approximately half of the duration observed in the previous conditions, suggesting a fatigue effect (Table 2, Figure 2).

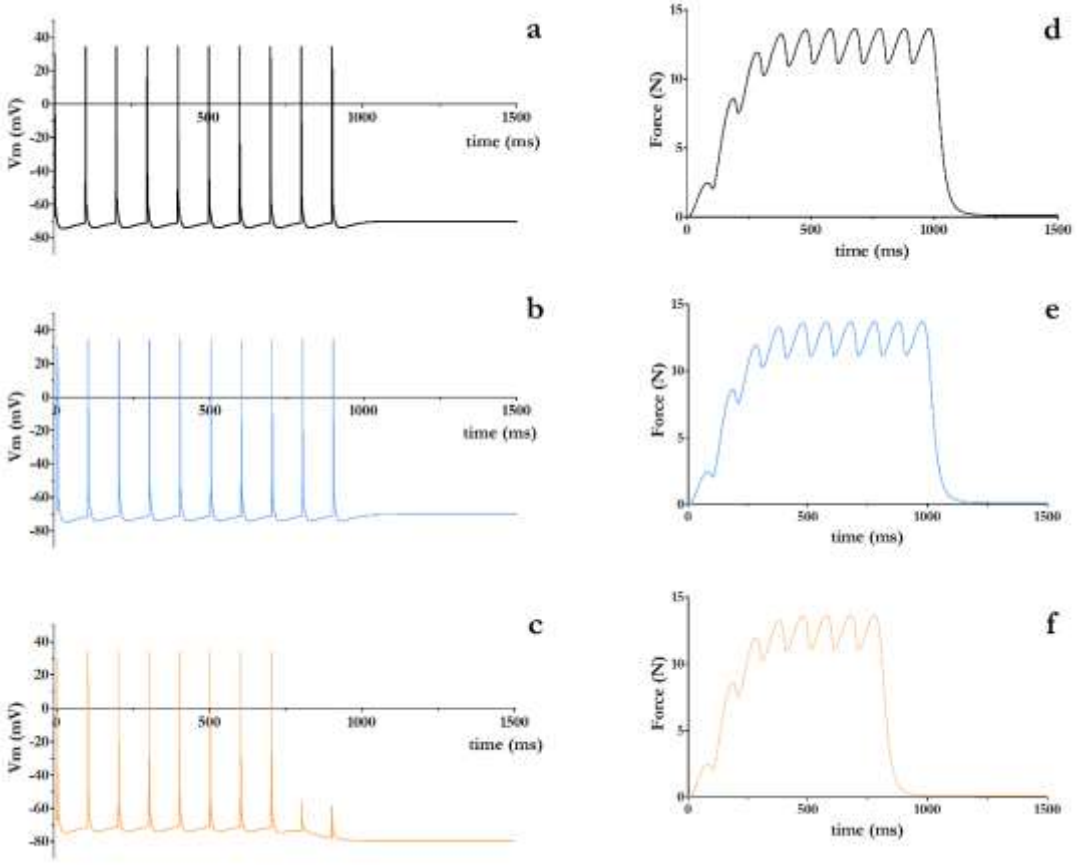

Figure 1. Motoneuron Membrane Potential and Muscle Force Generation at a Stimulation Frequency of 10 Hz. Motoneuron membrane potential without 5-HT receptors (a), with 5-HT1a and 5-HT2a receptors during physiological 5-HT release (b), and with 5-HT1a and 5-HT2a receptors during high 5-HT release (c).(d-f) Muscle force generated by the muscle innervated by the corresponding motoneuron in (a), (b), and (c), respectively.

Neuronal Activity and Muscle Force Generation with 100Hz Stimulation

At 100 Hz, significant differences were observed in all comparisons ($p < 0.0001$). The motoneuron with 5-HT receptors under physiological 5-HT release (b) showed an increase in excitability compared to the condition without receptors (a), whereas high 5-HT release (c) resulted in the inhibition of the response compared to both previous conditions (Table 1, Figure 3). Regarding muscle force during 100 Hz stimulation, the increased activation of 5-HT (c) generated a peak force similar to conditions (a) and (b), but with a significant decrease in the ability to maintain contraction over time ($p < 0.0001$). A "fatigue" effect was observed, where the initial force was high but rapidly declined (Table 2, Figure 3).

Discussion

The results of our study demonstrate that the electrical activity of motoneurons and muscle force generation are significantly influenced by the presence of 5-HT receptors and the concentration of serotonin in synaptic and

adjacent environments. The implications of these findings are discussed below in the context of existing literature and their relevance to motor control and certain neuromuscular pathologies.

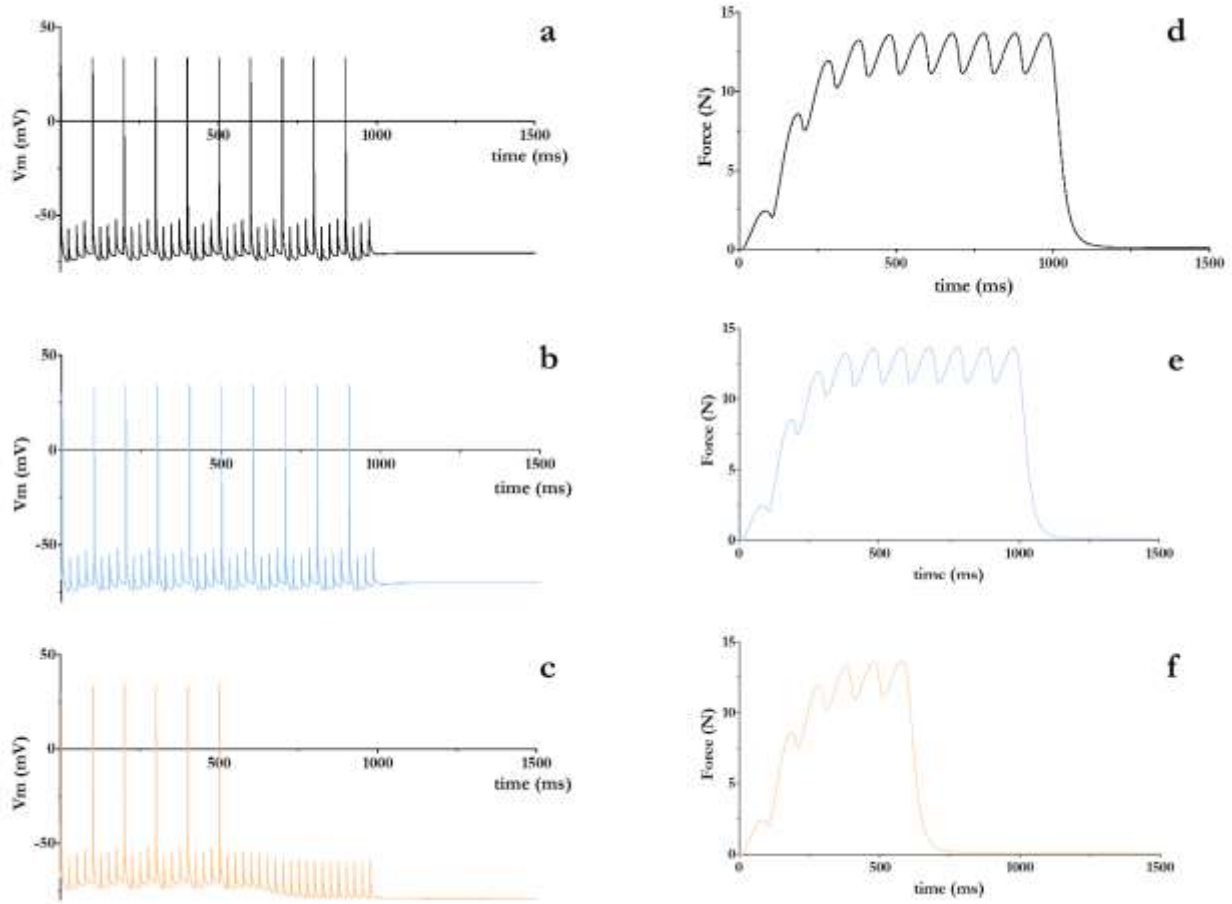

Figure 2. Membrane Potential of Motoneurons and Muscle Force Generated with 40 Hz Stimulation Frequency. Membrane potential of motoneurons without 5-HT receptors (a), with 5-HT1a and 5-HT2a receptors during physiological 5-HT release (b), and with 5-HT1a and 5-HT2a receptors during high 5-HT release (c). (d-f) Force generated by the muscle innervated by the corresponding motoneuron in (a), (b), and (c), respectively.

Serotonergic Modulation of Motoneuronal Excitability

Our results showed that physiological 5-HT release increases motoneuron excitability, particularly at higher stimulation frequencies (40 Hz and 100 Hz). This effect is consistent with previous studies that have shown serotonin acts as an excitatory neuromodulator in motoneurons, facilitating action potential generation through the activation of 5-HT2a receptors [12, 13]. However, high concentrations of 5-HT result in a decrease in excitability, suggesting an inhibitory effect possibly mediated by excessive activation of 5-HT1a receptors, known for their role in neuronal hyperpolarization [8, 14].

Implications for Muscle Force Generation and Fatigue

The generated muscle force was directly correlated with motoneuron activity. At a 10 Hz stimulation frequency, physiological 5-HT release did not significantly alter the force produced, suggesting that serotonin has no notable impact on muscle contraction under low-frequency stimuli. However, high 5-HT concentrations reduced the duration of contraction, indicating the possible induction of muscle fatigue. At higher stimulation frequencies (40 Hz and 100 Hz), physiological 5-HT release increases motoneuron excitability, which could facilitate a more sustained and powerful muscle response. However, exposure to high 5-HT concentrations results in a decreased

ability to maintain contraction over time, indicating a fatigue effect. This phenomenon could be explained by overstimulation of serotonergic receptors, leading to desensitization or disruption of motoneuron ionic homeostasis [4].

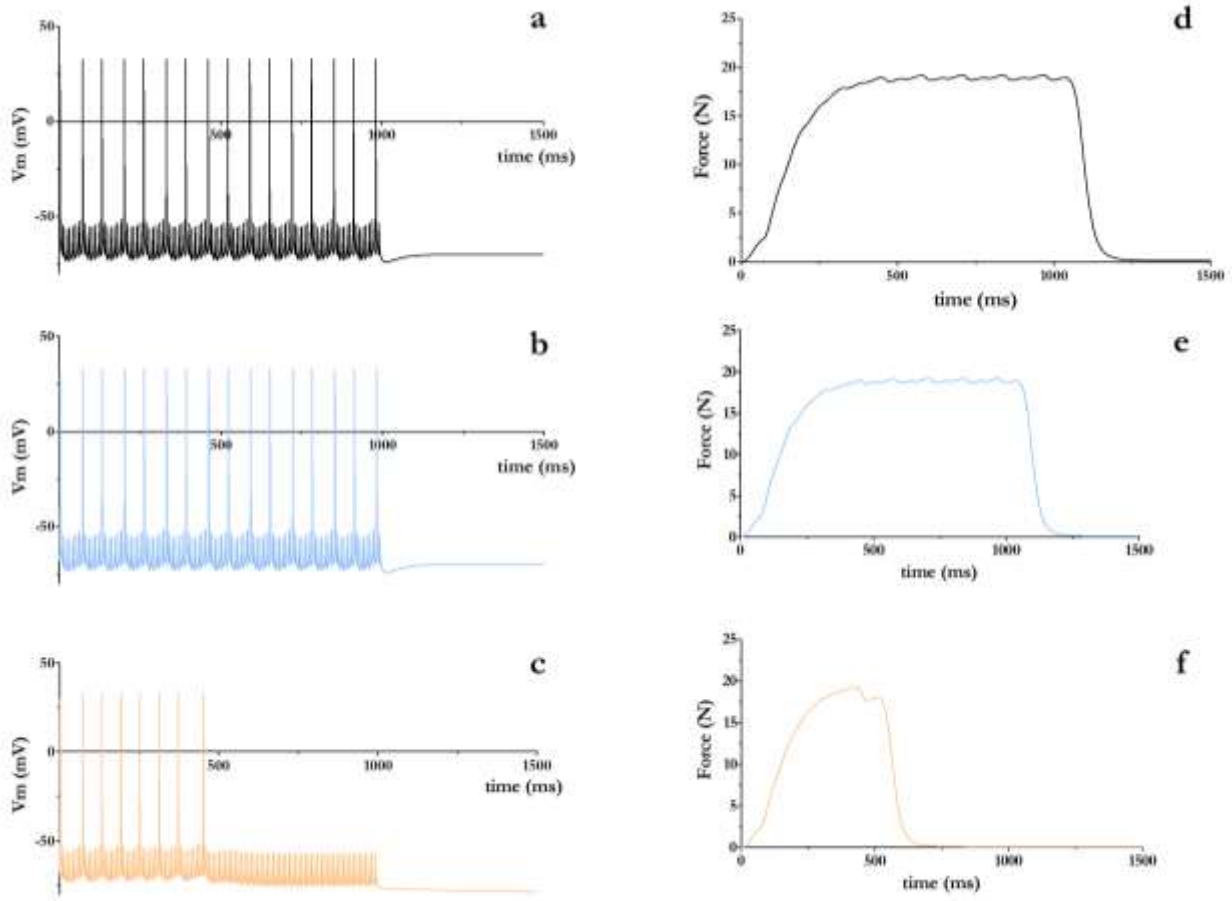

Figure 3. Membrane Potential of Motoneurons and Muscle Force Generated with 100 Hz Stimulation Frequency. Membrane potential of motoneurons without 5-HT receptors (a), with 5-HT1a and 5-HT2a receptors during physiological 5-HT release (b), and with 5-HT1a and 5-HT2a receptors during high 5-HT release (c). (d-f) Force generated by the muscle innervated by the corresponding motoneuron in (a), (b), and (c), respectively**.**

Comparison with Experimental Studies and Model Validation

The findings from our computational model align with those of experimental studies that have investigated the role of serotonin in motor function. It has been reported that modulation of 5-HT receptors can influence muscle fatigue resistance, supporting the idea that a proper balance in serotonergic signaling is essential for maintaining motor function [3, 4, 15]. The consistency between our modeling results and the existing experimental data suggests that the developed model is a valid tool for exploring the influence of serotonin on neuromuscular function. This model can serve as a foundation for future research aimed at understanding the underlying mechanisms of muscle fatigue and developing therapeutic interventions that modulate serotonergic signaling to improve muscle performance and recovery after injury. Furthermore, it allows the study of different ionic currents underlying neuromuscular excitability and inhibition.

Study Limitations and Future Perspectives

Although our model provides valuable insights into the interaction between serotonin and neuromuscular functions, it is important to acknowledge its limitations. Computational modeling simplifies the complexity of the neuromuscular system and may not capture all the variables present in a living organism. Therefore, additional experimental studies are required to validate and refine this model. Future research should focus on exploring interactions with other neurotransmitters, such as dopamine, adrenaline, and GABA, to understand how they affect motor function and fatigue resistance.

Conclusion

The proposed model is consistent with the results of the experimental studies. Our findings suggest that fluctuations in serotonin concentration can affect muscle force generation, providing a model for studying fatigue induced by this neurotransmitter.

List of Abbreviations: Not applicable.

Author Contributions: JM and DP performed the experiments. JM participated in the design of the study and performed statistical analysis. JM and DP coordinated and drafted the manuscript. All authors have read and approved the final manuscript.

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