Discovery on the Profile of Postsynaptic Potentials in Human Motoneurons

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Discovery on the Profile of Postsynaptic Potentials in Human Motoneurons

Koc University

Graduate School of Health Sciences

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ABSTRACT

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Postsynaptic potential (PSP) investigations on humans are based on indirect recordings since it is impossible to record directly from human motoneurons. This indirect estimation of a PSP can be acquired by recording the muscle activity with electromyography (EMG) in response to stimulation of specific sensory fibers. On the other hand, it is possible to use direct methods on in vitro animal models by injecting predetermined PSPs into regularly discharging motoneurons and evaluate their output by the methods used in human studies. In this thesis, these direct recordings from animal studies have been integrated into indirect human studies to reveal the human PSP profile more clearly.

The animal data were collected from a previous research with permission. In that study, current waveforms were injected into regularly discharging hypoglossal motoneurons to mimic PSPs in rat brain slices. The motoneuron background discharge rate and PSP duration output were procured from these experiments to investigate their correlation. The duration of the PSPs were detected with a discharge-based method Peristimulus Frequencygram (PSF) since it was shown to estimate the underlying PSPs more correctly than probability-based methods, which are used widely in the literature. A significant correlation was found between the background discharge rate and the duration of EPSP and IPSPs with linear regression. Since the injected PSP duration was predetermined in these studies, the motoneuron background discharge rate that delivers the exact PSP duration was obtained from linear regression analysis. This discharge rate was thought to reflect the PSP duration more correctly in human experiments.

A similar discharge rate versus PSP duration correlation plot was constructed in three EPSP and three IPSP experiments. H-reflex, transcranial magnetic stimulation (TMS) induced motor evoked potential (MEP), and masseter stretch reflex experiments were performed to study excitatory postsynaptic potential (EPSP) profiles; and Renshaw inhibition, masseter inhibition, and cutaneous silent period (CSP) experiments were used to study inhibitory postsynaptic potential (IPSP) profiles. The duration of the PSPs was also found by PSF together with cumulative sum (CUSUM) analysis to indicate the timing of stimulus-induced significant events accurately. The background discharge rate effect on the duration of PSP was found significant only in IPSP experiments. The motoneuron background discharge rate acquired from rat brain slice studies was used in human regression analyses to estimate the genuine IPSP duration in human. The estimated IPSP duration was found to be 192.10 ms in CSP, 43.94 in Renshaw, and 191.20 ms in masseter inhibition experiments.

The background effect on EPSP duration was not significant, probably because obtaining the falling phase of the EPSP was a great challenge. Therefore, more experiments are necessary with rearranged stimulus intensities.

The combination of the direct and indirect methods was a novel approach and suggests more understanding of synaptic connections between neurons; thus, it may procure more insight into the functioning of neuronal circuits in normal and pathological conditions.

ÖZETÇE

İnsan Motornöronlarında Postsinaptik Potansiyellerinin Profilinin İncelenmesi

Betilay Topkara Arslan
Nörobilim Doktorası - 7 Temmuz 2021

İnsan çalışmalarında motornöronlardan direkt kayıt alınması imkansız olduğundan postsinaptik potansiyel (PSP) çalışmaları indirekt kayıtlara dayanmaktadır. PSP profili insanda, duyusal fiberlere uyarı verilirken kas yanıtının elektromiyografi (EMG) ile ölçülmesinden yola çıkılarak ölçülür. Ancak in vitro hayvan modellerinde önceden belirlenmiş PSP'leri düzenli atan motornöronlara enjekte ederek direkt kayıt yapabilmek ve deneylerinin çıktısını insan çalışmalarında kullanılan metodlarla analiz etmek mümkündür. Bu tezde hayvanlardan alınan direkt kayıtları insan çalışması içerisinde kullanarak postsinaptik potansiyel profilini daha net bir şekilde ortaya koymak hedeflenmiştir.

Bu tezde kullanılan hayvan çalışması datası daha önce yapılan bir çalışmadan izin alınarak kullanılmıştır. Bu hayvan çalışmasında düzenli atan sıçan hipoglossal nöronlarına PSP oluşumunu taklit edecek bir akım enjekte edildi. Motornöron atım hızı ve PSP süresi arasındaki ilişkiyi incelemek üzere çalışmadan bu data alındı. PSP süresi atım hızını baz alan bir analiz yöntemi olan peristimulus frekansgram (PSF) ile bulundu. Bu metod PSP profilini bulmada literatürde sıklıkla kullanılan olasılık bazlı metodlara göre çok daha doğru sonuç gösterdiği için kullanılmıştır. Linear regresyon analizi ile motornöron atım hızı ile EPSP ve IPSP süresi arasında anlamlı bir ilişki bulunmuştur. Sıçan çalışmalarından alınan veride enjekte edilen PSP süresi tam olarak bilindiğinden, lineer regresyon analizinde buna karşılık gelen motornöron atım sayısı hesaplanabilmektedir. Direkt kayıtlardan alınan bu atım frekansının insan çalışmalarında PSP süresinin daha doğru olarak saptanmasına katkısının olacağı düşünülmüştür.

İnsanda üç tane eksitatör post sinaptik potansiyel (EPSP) ve üç tane inhibitör post sinaptik potansiyel (IPSP) deneyi tasarlanmış ve hayvan deneyleri ile aynı şekilde analiz edilerek motornöron atım sıklığı ve PSP süresi arasındaki korelasyon incelenmiştir. EPSP profilini araştırmak amacıyla masseter uzama refleksi, transkraniyal manyetik stimulasyon (TMS) ve H-refleks deneyleri; IPSP için ise Renshaw inhibisyonu, masseter inhibisyonu ve kutanöz sessiz periyod deneyleri tasarlanmıştır. Burada da PSP süresi PSF metodu ile bulunmuş ve bununla beraber kümülatif toplam analizi kullanılarak uyarının indüklediği anlamlı olaylar daha net ortaya çıkarılmıştır. Motor ünite atım frekansı, PSP süresi arasındaki ilişki sadece IPSP deneylerinde anlamlı çıkmıştır. Sıçan çalışmasından elde edilen atım frekansı kullanılarak IPSP süresi kutanöz sessiz periyod deneylerinde 192.1 ms , Renshaw'da 43.94 ms ve masseter inhibisyon çalışmalarında 191.20 ms olarak hesaplanmıştır.

Motor nöron atım hızı ve EPSP süresi arasında anlamlı ilişki bulunamamasının temel sebebinin bu çalışmadaki deneylerde EPSP'nin düşen fazının saptanamaması olduğu düşünülmektedir. Bu sebeple uyarı şiddetlerinde düzenlemeler yapılarak daha fazla deneyin yapılması planlanmaktadır.

Direkt ve indirekt metodların birleştirilmesi yeni bir yaklaşımdır ve nöronlar arasındaki sinaptik ilişkilerin daha iyi anlaşılmasına önayak olarak nöral devrelerin sağlıklı ve patolojik durumlardaki fonksiyonlarının daha iyi incelenmesine yardımcı olması öngörülmektedir.

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ABBREVIATIONS

EPSP	Excitatory Postsynaptic Potential
IPSP	Inhibitory Postsynaptic Potential
SMU	Single Motor Unit
MN	Motoneuron
PSTH	Peristimulus Time Histograms
PSF	Peristimulus Frequencygram
SEMG	Surface Electromyography
IM-EMG	Intramuscular Electromyography
TMS	Transcranial Magnetic Stimulation
CSP	Cutaneous Silent Period
ST	Sensory Threshold
APB	Abductor Pollicis Brevis
TA	Tibialis Anterior
FDI	First Dorsal Interosseous
MN	Motoneuron
MEP	Motor Evoked Potential
RC	Renshaw Cell
RI	Renshaw Inhibition
FT	Firing Threshold
AP	Action Potential
AHP	After Hyperpolarization

CHAPTER 1
INTRODUCTION

Synaptic connections between neurons are critical factors to understand the functions of neural circuits. Measuring postsynaptic potentials (PSPs) has been an effective tool in studies for years to evaluate neuronal circuitries' physical structure and function. Electrophysiological recordings have been performed directly on animal preparations by intracellular recordings to record PSPs amplitude, latency, and duration. However, animals are under anesthesia during those recordings, and anesthesia has been known to alter the functions of neuronal circuitries (Yuan et al., 2016). In contrast, direct recording on human subjects is impossible, and, therefore, indirect recordings have been used to obtain information about neuronal circuitries in human subjects. Although indirect recordings are subject to methodological errors (Turker & Powers, 2003), indirect human experiments are free from anesthetic effects, and hence they are treasured.

In PSP studies on humans, specific sensory receptors are stimulated via electrical or mechanical means, and neuronal responses to those stimuli are recorded from muscles via electromyography (EMG) during slight contraction of the related muscle. Surface electromyography (SEMG) and intramuscular electromyography (IM-EMG) are used to acquire the motoneuron responses to the stimuli. The literature's classical analysis techniques are full-wave rectification of SEMG signals around the stimulus and building spike-triggered averages (STA) of the data. For the IM-EMG signals, peristimulus time histograms (PSTH) are usually built. PSTH estimates spike occurrence probability in the postsynaptic cell around the stimulation time (Ashby & Zilm, 1982a). Although PSTH is an efficient method to demonstrate PSP initiation, it contains substantial synchronization errors in showing PSPs' actual characteristics, especially in terms of duration and profile (Miles, Türker, & Nordstrom, 1987; Turker & Cheng, 1994; Turker, Yang, & Scutter, 1997). However, PSTH is still commonly used to obtain PSP characteristics in electrophysiological studies. A novel approach has been developed to diminish those errors based on the discharge rate of the motor units, peristimulus frequencygram (PSF) (Turker & Cheng, 1994; Turker & Powers, 1999). Since the PSF approach uses instantaneous discharge rate, it minimizes the

synchronization and count-based errors, also proven by rat brain slice studies (Turker & Powers, 2003). Brain slice experiments have shown that the PSTH is more accurate to determine the latency of the PSPs, and the PSF is more efficient in obtaining PSPs' duration and general profile (Turker & Powers, 2005). Therefore, in this study, both methods have been used to show the actual characteristics of PSPs in human studies. Latency of the PSP was calculated from PSTH, and its duration was calculated from PSF analysis. Additionally, the Cumulative Sum (CUSUM) method has been used for both PSTH and PSF methods to pinpoint significant events more accurately (Brinkworth & Turker, 2003).

The main aim of this thesis was to combine animal and human experiments to find the characteristics of PSPs in human motoneurons. To achieve this aim, we used the animal data obtained in a previous study that used current waveforms to mimic PSPs in regularly discharging hypoglossal motoneurons in rat brain slices (Turker & Powers, 2003). In these studies, injected PSPs prompted alterations in the discharge output of the hypoglossal motoneurons in brain slices. These alterations in discharge rates were illustrated using both the PSTH and PSF analysis. In these experiments, it was discovered that the PSP durations calculated using the PSTH and PSF analyses did not always match the exact duration of the injected PSPs but changed depending on the motoneuron discharge rate. However, since the injected PSP duration was predetermined, the motoneuron background discharge rate that delivers the exact PSP duration could be obtained in these studies using a correlation plot between the discharge rate and PSP duration.

This thesis utilized the findings from the rat brain slice experiments to discover the exact PSP durations in human motoneurons. A similar discharge rate versus PSP duration correlation plot was constructed in three EPSP and three IPSP experiments to achieve the aim. H-reflex, transcranial magnetic stimulation (TMS) induced motor evoked potential (MEP), and masseter stretch reflex experiments were performed to study excitatory postsynaptic potential (EPSP) profiles; and Renshaw inhibition, masseter inhibition, and cutaneous silent period (CSP) experiments were used to study inhibitory postsynaptic potential (IPSP) profiles. PSTH-CUSUM and PSF-CUSUM, respectively, were also used to determine the latency and duration of the EPSP/IPSP. The spike-triggered averaging (STA) method was used to analyze multi motor unit (MMU) and SEMG data. CUSUMs of the SEMG and multi-unit data were also

obtained in all experiments to compare the current findings with that of the findings of the published literature.

1.1. Postsynaptic Potentials

Presynaptic neurons release a neurotransmitter from the terminal button at the end of an axon into the synaptic cleft to respond to a neuronal impulse. That neurotransmitter binds to the specific receptor in the postsynaptic terminal's plasma membrane, a neuron, or a muscle cell (in neuromuscular junction). A temporary electrical change occurs in the postsynaptic membrane called postsynaptic potential (PSP) (Mark F. Bear, 2016). This potential arises from the binding of neurotransmitters to specific membrane receptors on the postsynaptic density. Postsynaptic membrane receptors are classified into two main categories: ionotropic (transmitter-gated ion channels) and metabotropic receptors (G-protein coupled receptors). Depending on the permeability of these receptors to ions, the postsynaptic potentials can be excitatory or inhibitory. For example, if open channels are permeable to Na+ ions, then the cell will be depolarized. Depolarization of the postsynaptic membrane brings membrane potential near the threshold (-65 mV), called excitatory postsynaptic potential (EPSP) (Fig. 1.1). Glutamate-gated ion channels and acetylcholine-gated (ACh-gated) ion channels activation results in EPSPs

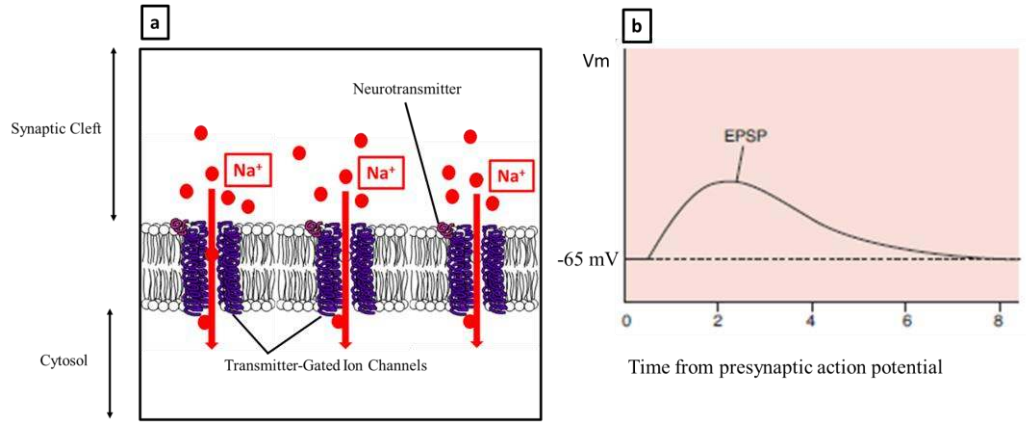


Figure 1.1. Generation of an EPSP.

(a) The neurotransmitters bind to transmitter-gated ion channels in the postsynaptic membrane. Na+ influx through those channels causes depolarization. **(b)** Change in the membrane potential (Vm) is called EPSP, which is recorded by a microelectrode in the cell (The figure was modified from Mark F. Bear (2016).

On the contrary, if open channels are permeable to K+ or Cl- ions, then the inside of the cell becomes more negative, which leads to hyperpolarization. In this case, membrane potential gets away from the threshold, termed inhibitory postsynaptic potential (IPSP) (Fig 1.2). Gamma-Aminobutyric Acid-Gated (GABA-gated) or glycine-gated ion channel activation causes IPSPs (Tortora & Derrickson, 2018).

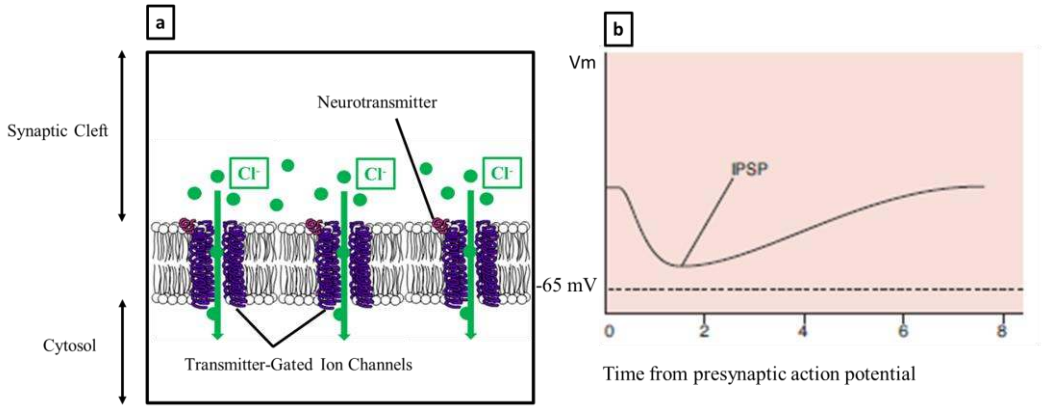


Figure 1.2. Generation of an IPSP.

(a) The neurotransmitters bind to transmitter-gated ion channels in the postsynaptic membrane. In the case of Cl- influx through those channels causes depolarization in the postsynaptic membrane. **(b)** Change in the membrane potential (Vm) is called IPSP, which is recorded by a microelectrode in the cell (The figure was modified from Mark F. Bear (2016).

1.2. Postsynaptic Potential (PSP) Studies

PSPs can not be recorded directly from human motoneurons; however, their size, shape, initiation, duration characteristics can be inferred from specialized electrophysiological experiments. These experiments include stimulation of peripheral or corticospinal fibers during the slight voluntary activity of single or more motoneurons. SEMG and IM-EMG recordings are used for the representation of these motoneurons in response to the given stimuli. These results are investigated via probability-based and rate-based methods.

The probability-based methods estimate the probability of spike occurrence in the postsynaptic cell from the stimulus initiation. The oldest probability-based method is averaging the full-wave rectified SEMG around the stimulus time, i.e., the spike-triggered averaging (STA) method. Full-wave rectification adds the SEMG signal under the baseline to the signal above the baseline to make all signals positive (Fig.1.3).

Averaging rectified SEMG around the stimulus time reveals the inhibitory or excitatory neuronal pathways that connect the stimulated site to motoneurons. Using this method, peaks that occurred in response to the stimulation are inferred as excitation and troughs considered inhibition.

Although this method presents a general view of the neuronal networks that connect the stimulated site to a group of motoneurons, it fails to measure connections to individual motoneurons (Poliakov & Miles, 1992). Since SEMG provides an overall representation, it is unlikely to obtain if different motoneurons in the motoneuron pool respond differently to the stimulus (Miles, 1997).

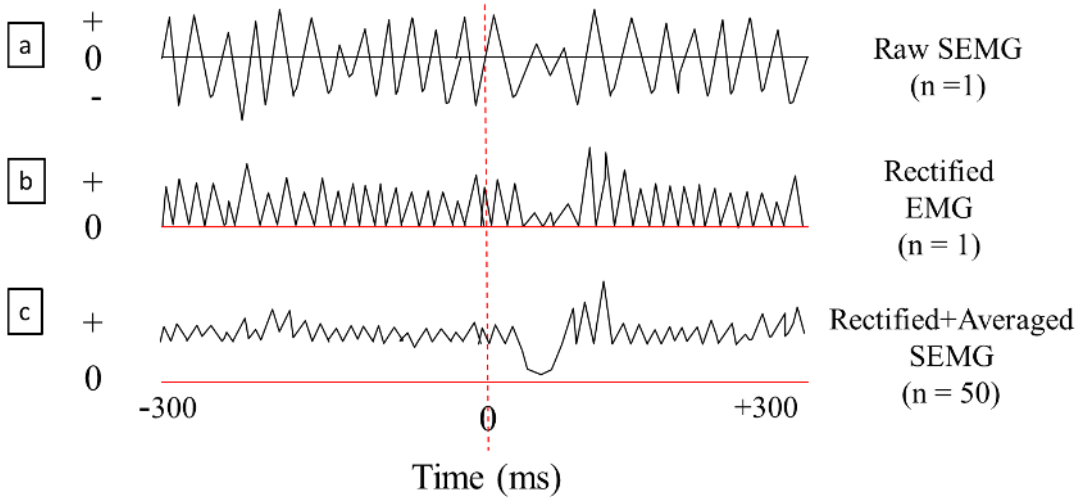


Figure 1.3. SEMG Analysis Representation.

(a) Raw SEMG signal. **(b)** Rectified SEMG around the stimulus (n=1) **(c)** Rectified and averaged SEMG signal showing inhibition response (n=50). The figure was modified from Türker (2021).

Single motor unit recordings (SMU) were thought to solve these limitations since quantification of responses can be performed more accurately. The subjects are asked to gently contract his/her muscle to reveal spike trains while delivering inhibitory or excitatory stimuli. The effect of those stimuli on discharging single motor units is evaluated. Intramuscular EMG (IM-EMG) recordings have been shown to identify postsynaptic events more precisely than SEMG recordings. Peristimulus time histograms (PSTHs) were proposed to quantify the IM-EMG recordings to investigate

PSPs (Moore, Segundo, Perkel, & Levitan, 1970). PSTH shows the possibility of a motoneuron spike occurrence as a function of time from the stimulus onset. Therefore it is a probability-based method; it counts the spikes and superimposes them around the stimulation (Fig. 1.4a). The significant point here is that PSTH needs the prestimulus values to calculate the effect of the stimulus.

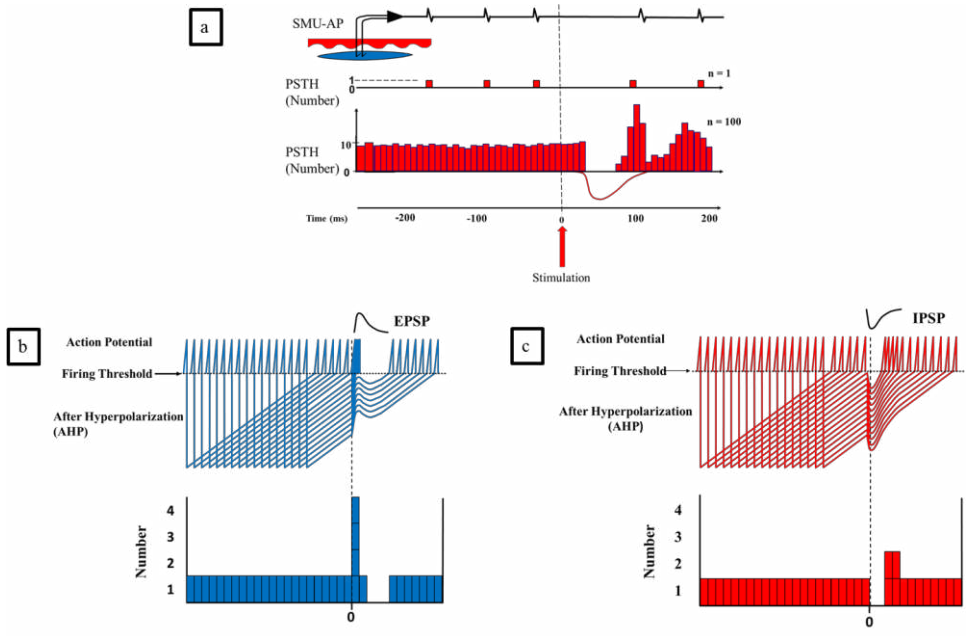


Figure 1.4. Construction of the PSTH.

(a) Each SMU spike was converted into accepted pulses. Peristimulus time histogram (PSTH) indicates them as counts strictly at each bin they occur (second trace from the top). The spike number counted and superimposed around the stimulation. The red trace in PSTH represents IPSP **(b).** Excitatory stimulation brings the spikes forward. They appear before their predetermined time intervals (phase-advanced spikes) and accumulate there, which is interpreted as an excitatory event in PSTH. **(c)** Inhibitory stimulation delays spike occurrence; the gap in the figure represents inhibition in PSTH. These phase delayed spikes are gathered after inhibition which is interpreted as excitation. The figure was modified from Türker (2021).

An excitatory stimulus tends to bring the action potentials forward, meaning the motoneuron fires earlier than is anticipated (1.4b). The spikes that fire earlier due to the stimulation accumulates, and that accumulation constitutes the peaks inferred as excitation. Conversely, an inhibitory stimulus takes the action potentials farther;

thereby, the motoneuron fire later than expected. That causes a blank period in the histogram, and that trough is considered to be an inhibition (1.4c).

To sum up, phase-advanced or delayed spikes occur due to synaptic effects on the spike probability. These changes in spike probability generate auto-correlation (synchronization) of spikes which are seen as secondary and tertiary peaks or troughs in the histogram. The problem here is that these synchronization-based events are interpreted as excitatory or inhibitory incidents, and long and complex neuronal pathways are proposed to explain such late occurrences. A discharge-rate-based approach, peristimulus frequencygram (PSF), was suggested to reduce these errors (Turker & Cheng, 1994; Turker & Powers, 1999). PSF approach uses superimposition of the discharge rates of single motor units instead of counting the spike occurrence around the stimulus time; thus, it is less contaminated by count- and synchronization-related errors (Turker & Powers, 2003).

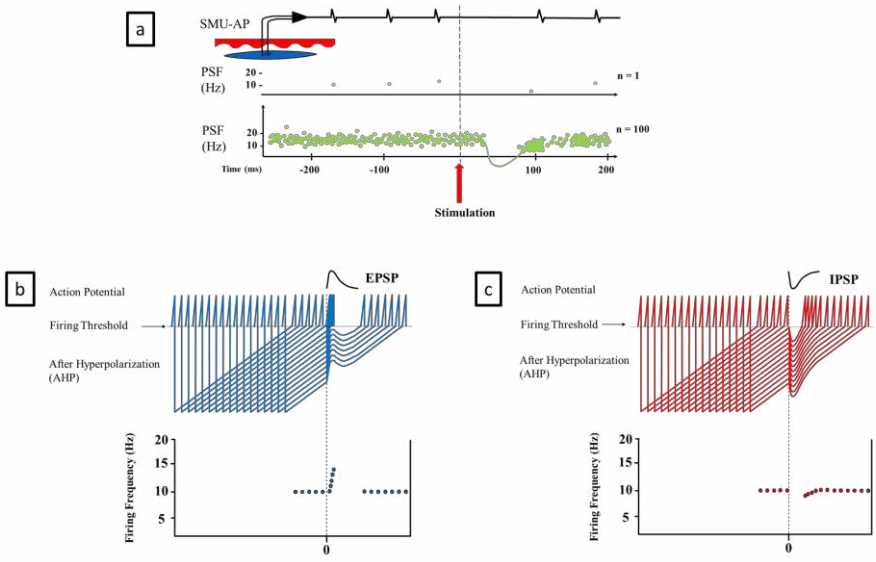


Figure 1.5. Construction of the PSF.

(a) SMUs were converted into accepted pulses to building the peristimulus frequencygram (PSF). PSF points them out as instantaneous discharge frequency. PSF shows superimposition of the discharge rates of single motor units around the stimulus. **(b)** An excitatory stimulus increases the firing rate of the SMUs, **(c)** An inhibitory stimulus decreases the discharge rates of the units. Modified from Türker (2021).

PSF depicts the instantaneous discharge frequency of SMUs against stimulus time (Fig 1.5a). An excitatory stimulus increases the discharge frequency of the units. This increase constitutes the initiation phase of EPSP (Fig 1.5b). The critical point here is the

inhibition seen after excitation in PSTH is not seen in PSF. On the contrary, inhibitory stimulus decreases the discharge rates of units (Fig 1.5c).

A neuron's discharge rate represents the net currents arriving at its soma (Lee, Kuo, Jiang, & Heckman, 2003; Powers, Robinson, Konodi, & Binder, 1992); therefore, any change in the poststimulus discharge rate points out a net synaptic input. (Turker, Yang, & Scutter, 1997). Due to that assumption, the PSF approach reflects the PSP profile more accurately than the PSTH method. This hypothesis is tested by Turker and Powers (1999) with direct recordings on hypoglossal motoneurons. They injected current transients to evoke repetitive discharges in hypoglossal motoneurons. A suprathreshold injected current step integrated with superimposed noise to simulate synaptic signal during physiological activation. The responses of motoneurons to that injected currents were evaluated via PSTH and PSF methods. Comparison of PSTH and PSF showed that PSF is more accurate in revealing the PSP profile (Fig 1.6). PSTH results depict silent periods after excitatory stimulation (Fig 1.6a); however, PSF shows that inhibition (I1) in PSF is the falling phase of the EPSP (1.6c). The followed secondary and tertiary peaks and troughs in the PSTH arise from synchronization errors associated to the stimuli. A similar pattern is seen for the inhibitory stimulations. The excitation (E1) presented in PSTH after the inhibition is actually rising phase of the IPSP (1.6b). The following peaks and troughs are also synchronization errors. The major problem here is that these errors are still interpereted as inhibition or excitation in several current studies (Ganczer et al., 2017; Moscato et al., 2019; Zare et al., 2018)

Although PSF is a practical approach to reveal the PSP profile, it has limitations. For example, it fails to demonstrate the PSP trajectory entirely if the discharge rate is too low (Turker & Powers, 2005). Additionally, the PSTH approach is more accurate in obtaining the initiation of PSPs (Turker & Powers, 2003).

Therefore, using PSTH and PSF in combination would bring forth the most accurate results (Turker & Powers, 2005). In this study, PSTH is used to obtain the latency of the PSPs, and PSF is to acquire the duration. Additional analysis is performed to procure more sensitive results. Cumulative sum (CUSUM) analysis is used with PSTH and PSF to reveal subtle but persistent changes in the PSPs (Brinkworth & Turker, 2003). CUSUM method is composed of repetitive calculations of the calculating sum of residuals, and it obtains significant changes by identifying the deviations from a reference point (Koepcke, Ashida, & Kretzberg, 2016).

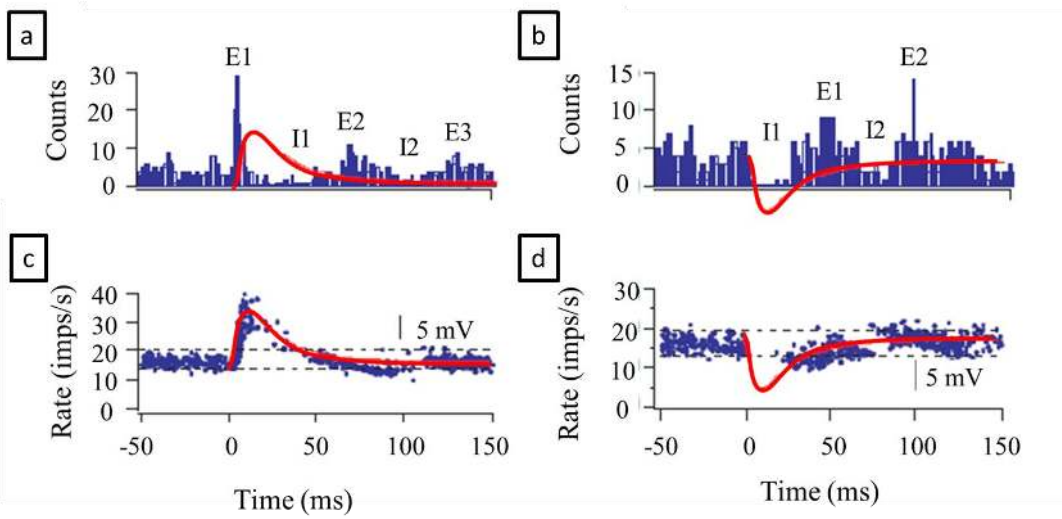


Figure 1.6. PSTH versus PSF in response to predetermined PSPs.

(a) PSTH response to an EPSP with a rise time of 5 ms, generated by current transient injection to repetitively discharging MN in rat brain slice and **(b)** similar IPSP injections. **(c)** PSF in response to the same EPSP and (d) IPSP injections. Modified from Turker and Powers (1999).

Hypoglossal motoneuron recordings also revealed the background discharge rate effect on the PSF profile (Fig 1.7). EPSP and IPSP amplitudes were kept constant (8 mV), and background discharge rates were varied to find the relation between the PSP characteristics (Turker & Powers, 1999). The presentation of the EPSP profile becomes more apparent as the background discharge rate is increased (Fig 1.7a). The peak change is almost identical in all background discharged rates; however, the falling phase of EPSP is invisible in lower background discharge rates. Similarly, IPSP becomes more evident in higher background discharge rates (Fig. 1.7b).

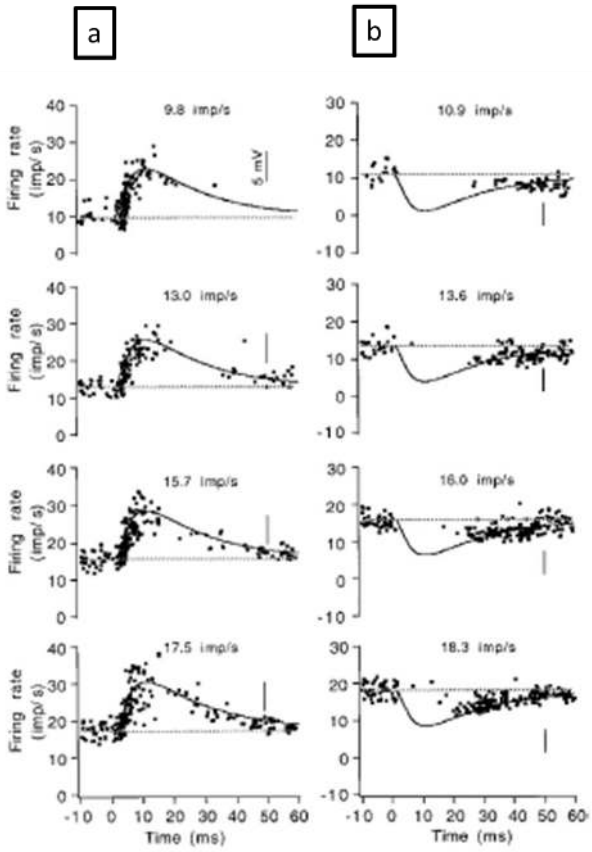


Figure 1.7. Effect of background discharge rate on the representation of the PSP profile
Same size **(a)** EPSP and **(b)** IPSP injection on a hypoglossal MN. Modified from Turker and Powers (1999)

In the light of all this information from slice recording experiments and human studies, it is thought that combining direct and indirect methods suggest a higher presentation of PSP features. Data collected from the rat hypoglossal neurons are used with permission in this thesis (Turker & Powers, 1999, 2003). The background discharge rate and PSP duration value are extracted from the study to evaluate discharge rate effect on PSP duration. (duration value is acquired from PSF analysis). Here, the injected current is predetermined (the duration and amplitude of it is known); thus, by extrapolating this value on this linear graph, the background discharge rate that illustrates the actual duration of the PSP can be found.

In human studies, three excitatory and three inhibitory experiments were performed to estimate features of PSPs by analyzing PSTH-CUSUM and PSF-CUSUM. These experiments were repeated with different frequencies, and background discharge rate-duration data were assessed by linear regression. Similar to the rat brain slice

experiments, the effect of background discharge rate on the PSP duration is examined by linear regression.

Studies on the human subjects to evaluate IPSPs in this thesis consist of the cutaneous silent period (CSP), masseter inhibition, and Renshaw inhibition experiments:

The cutaneous silent period (CSP) is produced by applying high-intensity (painful) electrical stimulation on the distal parts of the body. This stimulus creates an inhibition on the ongoing EMG activity, which is referred to as CSP (Inghilleri, Cruccu, Argenta, Polidori, & Manfredi, 1997; Uncini, Kujirai, Gluck, & Pullman, 1991), and it is thought to be a protective mechanism mediated by A-delta fibers (Kofler, 2003; Kofler, Leis, & Valls-Sole, 2019). In this thesis, CSP is generated by delivering high stimulus intensity to the little finger (ulnar nerve) and record the continuous activity via SMU and IM-EMG electrodes from the first dorsal interosseous muscle (FDI).

Masseter inhibitory reflex is also thought to be a protective reflex that is created by high intensity painful electrical stimulus around perioral regions, and it causes masseter muscle inhibition (Yemm, 1972a, 1972b). Strong electrical currents activate high-threshold afferents that transmit information about pain to the central nervous system; thereby, the inhibition occurs to prevent jaws and teeth from damaging (Türker, 2002). The high-intensity electrical stimulus was shown to create short-latency (early) and long-latency (late) inhibitions or a single long-lasting inhibition in masseter muscle (Miles et al., 1987; Uginčius, Yilmaz, Sebik, & Türker, 2017). Here in this study, we evaluate these short- and long-latency inhibitions as compound inhibition.

Renshaw inhibition (RI) is mediated by a specific type of inhibitory interneurons found in the gray matter of the spinal cord called Renshaw cells (RCs) (Eccles, Fatt, & Koketsu, 1954; Renshaw, 1941). RCs receive excitatory input from alpha-motoneuron collaterals, and they send inhibitory axons to synapse on the same alpha-motoneuron or/and an alpha-motoneuron in the same motoneuron pool (Katz & Pierrot-Deseilligny, 1999). In the experiments used in this thesis, motor fibers were delivered isolated stimulation (M-only stimulation) for the investigation of RC antidromic activation via MN collaterals (Kudina & Pantseva, 1988). This M-only stimulation approach based upon thickest axon stimulation which originate from the largest MNs, and recording the responses of voluntary activated SMUs concurrently. The Renshaw inhibition data presented in this thesis is a part of our published study (Ozyurt, Piotrkiewicz, Topkara, Weisskircher, & Turker, 2019).

EPSP investigations on human subjects represented in this thesis consist of Hoffman -Reflex (H-reflex), transcranial magnetic stimulation, and masseter stretch reflex experiments:

H-reflex is the electrical analog of the monosynaptic stretch reflex (Hoffman, 1910), and it is elicited by low-intensity electrical stimulation of the sensory fibers, which results in monosynaptic excitation of MNs (Knikou, 2008; Pierrot-Deseilligny & Mazevet, 2000). Many studies utilized H-reflex as a tool to study EPSP profile (Ashby & Zilm, 1982b; Birnbaum & Ashby, 1982; Miles, Turker, & Le, 1989). In the H-reflex experiments in this thesis, the tibial nerve was stimulated with low intensity from the popliteal fossa to activate Ia afferent reflex pathway and elicit H-reflex in the soleus muscle (Burke, 2016).

Transcranial Magnetic Stimulation (TMS) of the motor cortex induces a muscle action potential in the corresponding muscle, which is called motor-evoked potential (MEP) on electromyography (Barker, Jalinous, & Freeston, 1985). TMS was applied to corresponding areas of the motor cortex to induce MEPs of tibialis anterior (TA) and abductor pollicis brevis (APB) muscles to evaluate EPSPs.

Masseter stretch reflex is generated by tapping to the chin with a reflex hammer at a downward angle. The stimulus creates a brief, rapid stretch to jaw-closing muscles. A strong excitatory reflex takes place in these muscles with monosynaptic latency and it is believed that muscle spindles in jaw closer muscles are the main responsibles for this reflex (Türker, 2002).

In all human experiments duration of PSPs was obtained by PSF-CUSUM, and the correlation between the background discharge rate and duration was investigated. Since durations of PSPs were determined indirectly from human experiments, and they are affected by SMU discharge rate, we proposed in our early study that the actual duration of PSPs in a resting motoneuron can be obtained via linear extrapolation from the discharge rate (Ozyurt et al., 2019). More importantly, the background discharge rate data obtained from slice recordings integrated with human studies by extrapolating this value on the human experiment linear graph; thereby, the background discharge rate that represents the actual duration of the PSP can be detected.

Combining the direct and indirect methods suggests a novel approach for a better estimation of PSP duration, which would be beneficial to understand inhibitory and excitatory pathways in humans; it may provide more insight into the functioning of neuronal circuits. Also, after further improvements, it can be standardized on healthy

individuals; thereafter, it can be used on patients with neuromuscular diseases, neuropathies, or spinal cord injuries to rewire the nervous system in pathological conditions.

In addition to the results obtained from SMU recordings, Spike-triggered averaging (STA) method was also used to analyze multi-unit and SEMG data. CUSUMs of the SEMG and multi-unit data were used in all experiments to compare the current findings with that of the findings of the published literature.

CHAPTER 2
MATERIALS AND METHODS

Koç University Human Ethics Committee approved the experimental procedures in accordance with the Declaration of Helsinki (2017.066.IRB2.038). Human experiments were performed in Koç University Neurophysiology Laboratory, Koç University Hospital Motion Analysis and Cognition Laboratory, and Marmara University Pendik Training and Research Hospital Neurology Department. All subjects were filled up and signed the informed consent form (please see the form in Appendix). The subjects have no neuromuscular, neurological disease, or physiological problems that can affect the related experiments.

2.1. Recordings and Setup

Spike 2 version 7.20 software was used for data acquisition and analysis. Recordings were made via CED 3601 Power 1401 MKII digital to analog converter and CED 1902 Quad System MKIII amplifier. Digimeter DS7A was used for electrical stimulation.

Surface muscle activity was recorded by standard Ag/AgCl surface electrodes (Redline). Intramuscular muscle activity (IM-EMG) recording was performed by custom-made, tip-active Teflon insulated, silver fine wires (75 μm core diameter and 3T, Leico). The whole equipment list in this study is given in Table 2.1 and will be mentioned in the following sections.

The muscle interested is prepared before electrode placement. The skin is shaved (if necessary) and rubbed with sandpaper. Then the area is cleaned with 70% ethyl alcohol, and finally, conductance gel is applied to reduce the skin impedance. SEMG was recorded bipolarly; one electrode was placed on the belly of the muscle, and the other one was put in a 4 cm distance for optimal signal recognition (Tucker & Türker, 2005). This distance is less than 4 cm in smaller muscles (e.g., 2 cm for abductor pollicis brevis muscle). For grounding, a surface electrode located on a nonmuscular area nearby or a sterile lip-clip was used as a ground electrode (Türker, Miles, & Le, 1988).

The exact placements of the electrodes in different muscles will be explained in detail in the experimental designs section.

IM-EMG was recorded with tip-active Teflon insulated silver fine-wire electrodes. Bipolar wire electrodes inserted in the muscles in 25 G surgical needles. Immediately after, the needle was withdrawn and leave the fish-hooked wires inside the muscle. All electrodes were stabilized with surgical tape to reduce movement artifacts.

SEMG and IM-EMG were recorded with a 20000 Hz sampling rate. Raw data were collected for all experiments; however, SEMG was filtered with a frequency of 20-500 Hz, and IM-EMG with a 200-5000 Hz bandpass filter online since SEMG and IM-EMG signals were obtained optimally in those filter range. Signals were amplified 100 times (Bandpass filter and amplification differed from those values in some experiments, they will be explained in the following section).

Table 2.1. Equipment list used in this thesis.

Equipment	Experiment	Purpose	Features	Model	Company	Country
Software	All	Data Acquisition	Software	Spike 2-7.20	CED	UK
Amplifier	All	Signal Amplification	Quad System	1902 MKIII	CED	UK
Converter	All	Analog to digital converter	8 Channels	Power 1401 MKII	CED	UK
Electrode	All	SEMG recording	Ag/AgCl	50F	Redline	Turkey
Electrode	All	SMU recording	75 µm core diameter	Medwire-3T-Teflon insulated silver fine wire	Leico	USA
Stimulator	Masseter Inhibition, CSP, Renshaw, H-reflex Experiments	Electrical Stimulation	Constant current	Digitimer	DS7A	UK
Transducer	Renshaw Experiment	Force Recording	Linear to 196 N	LC1205-K020	A&D Co. Ltd.	Japan
Transcranial Magnetic Stimulator	TMS experiments	Brain Stimulation	Double cone coil electrode	Magstim 200^2	Magstim Co	UK
Ring Electrodes	CSP Experiments	Electrical stimulation of distal regions (fingers)	Double ring-shaped electrodes	Homemade	-	Turkey
Reflex Hammer	Masseter Excitation Experiments	To Induce Stretch Reflex	-	Homemade	-	Turkey
Lip Stimulator	Masseter Inhibition Experiments	Electrical Stimulation from the lip	Silver disc cathode, spring wire anode	Homemade	-	Australia

2.2. Experimental Designs

This thesis used rat brain slice experiments to reveal the exact duration of PSPs in human motoneurons. Data obtained from rat brain slice experiments were used with the implicit approval of Turker and Powers (1999). PSP duration versus discharge rate correlation was procured from these slice experiments. Human experiments were designed and analyzed to present corresponding data to acquire the exact duration of PSPs.

2.2.1. Human Experiments

Three excitatory postsynaptic potentials (EPSP) and three inhibitory postsynaptic potentials (IPSP) experiments were performed to investigate the precise profile of PSPs with the help of slice experiments. Transcranial Magnetic Stimulation (TMS) induced motor evoked potential, H-reflex, and masseter stretch reflex experiments were conducted to investigate EPSP profiles; cutaneous silent period (CSP), Renshaw inhibition, masseter inhibition experiments were to investigate IPSP profiled (Fig 2.2).

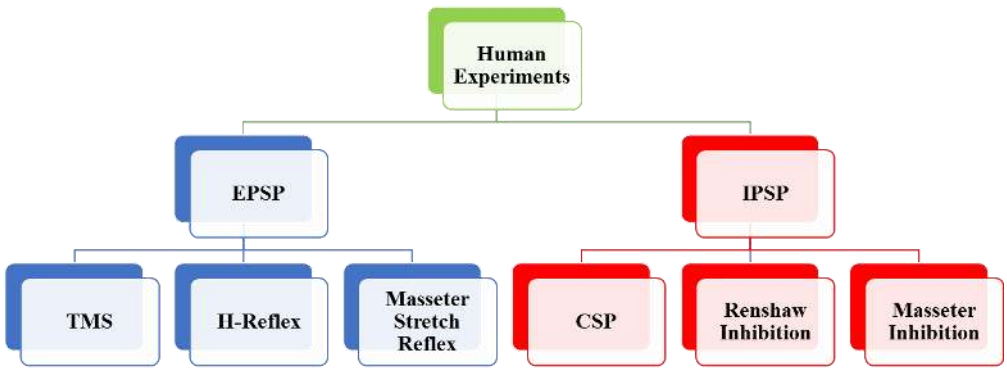


Figure 2.1. Human experiments performed in the thesis

EPSP experiments: Transcranial Magnetic Stimulation, H-Reflex, and Masseter stretch reflex; IPSP experiments: Cutaneous Silent Period (CSP), Renshaw Inhibition, and Masseter inhibition

Inhibitory Post Synaptic Potential (IPSP) Profile Estimation Experiments

An inhibitory stimulus causes synchronization of motor unit discharge rate by prolonging the interspike intervals (ISIs) (Kudina, 1980). These synchronized motor

units occur at a particular time point after the stimulus and accumulate there. In probabilistic analyses such as the PSTH and surface electromyography (SEMG), the absence/reduction of the number of motor units due to stimulus-induced lengthening of ISIs is inferred as inhibition and accumulation spikes after the period of reduced spike numbers as excitation. On the other hand, PSF analysis relies upon any stimulus-induced change in the discharge rate; a decrease in discharge rate is interpreted as inhibition and an increase in discharge rate as excitation. CUSUM is used both with PSF and PSTH to detect the postsynaptic potential (PSP) profile more precisely.

Cutaneous Silent Period (CSP) Experiments

Nine subjects participated in this study (5 males and four females; aged 28-53 years).

SEMG and IM-EMG are recorded from the right first dorsal interosseous (FDI) muscle. Subjects are asked to bring their thumb and index finger together and press to activate the FDI muscle. After the muscle preparation, procedures were performed as mentioned in **Section 2.1**. Two standard SEMG electrodes (Ag/AgCl) were placed on the FDI muscle 2 cm apart due to its small size. One ground electrode is placed on the metacarpal bone. The IM-EMG electrodes were inserted into the muscle in a 25 G surgical needle in between two SEMG electrodes. The needle was withdrawn immediately and left the fish-hooked wires inside the FDI muscle.

During the experiments, all subjects were seated in an upright position on a comfortable chair with their elbows at approximately 90 degrees of flexion. They received the electrical stimulus from their fifth digit via two ring-shaped adjustable electrodes placed around 2 cm apart. The fifth digit was chosen for stimulation location since it is located at the C8 dermatome, which includes cutaneous innervations that supply FDI muscle (Chiba et al., 2015) (Fig 2.2). Right-hand FDI muscle was used for all recordings.

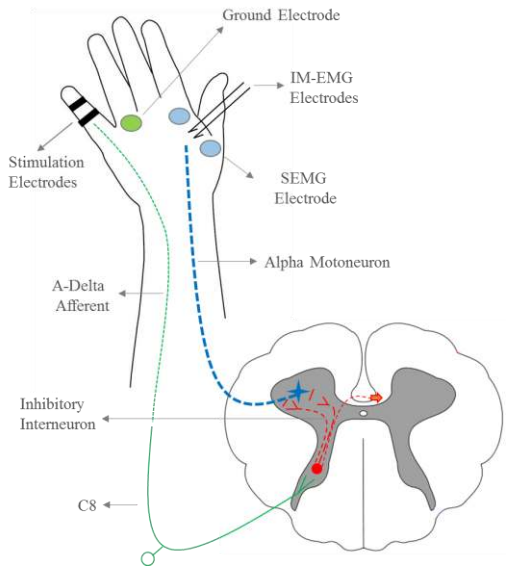


Figure 2.2. CSP Experiment electrode locations.

CSP was generated by delivering a high electrical stimulus from the fifth digit via ring electrodes; the response of the stimulus was recorded from FDI muscle via IM-EMG and SEMG electrodes. The ground electrode was placed on the metacarpal bone. The fifth digit was located on the C8 dermatome, which includes cutaneous innervations that supply FDI muscle.

Sensory threshold (ST) was found to define stimulus intensity. ST is described as the lowest stimulus intensity that subjects can perceive (Kofler, Leis, & Valls-Sole, 2019). The subjects received gradually increasing electrical stimulus starting from low intensities. 10 times ST were delivered to create a cutaneous silent period (CSP) during the experiments. 178 ± 82 (mean ± SD) stimuli are given in 1-2 seconds interval in 0.2 ms pulse width (Corsi et al., 2002; Kofler, Leis, & Valls-Sole, 2019; Shefner & Logigian, 1993).

Maximum voluntary contraction (MVC) was recorded three times with 10 seconds of rest after the sensory threshold was detected. Subjects were asked to contract their hands slightly to recruit their motor units. They have tracked their motor unit activity from a feedback monitor to activate only one SMU steadily. More than one motor unit activity is also accepted if they are separable via SMU analysis. The high-intensity electrical stimulus was started to deliver randomly when the SMU was active. Rectified and averaged SMU channel was monitored from a different screen during the

experiment to ensure the inhibition occurs with the stimulus. The analysis will be explained in detail in the 2.3 Analysis section.

Renshaw Inhibition Experiments

Ten subjects participated in this experiment (4 females, 6 males; aged 18-35 years). The Renshaw inhibition data presented in this thesis is a part of our published study (Ozyurt et al., 2019).

SEMG recordings were made from the right soleus and tibialis anterior (TA) muscles. For correct placement of the SEMG electrodes on soleus muscle, subjects were asked to perform plantar flexion. After preparations were completed as described in the 2.1 section, SEMG electrodes were placed on the lateral part of the soleus (4 cm apart). For TA muscle subjects performed dorsiflexion, electrodes were placed 4 cm apart after the preparation procedures were completed. Then, intramuscular electrodes were inserted into the soleus muscle as described above (section 2.1, Fig 2.3). A lip clip was used as a common ground for both SEMG and IM-EMG electrodes (Türker et al., 1988). SEMG recordings were bandpass filtered at 20-1000 Hz, and IM-EMG recordings with at 100-10000 Hz. Both were sampled at 20000 Hz.

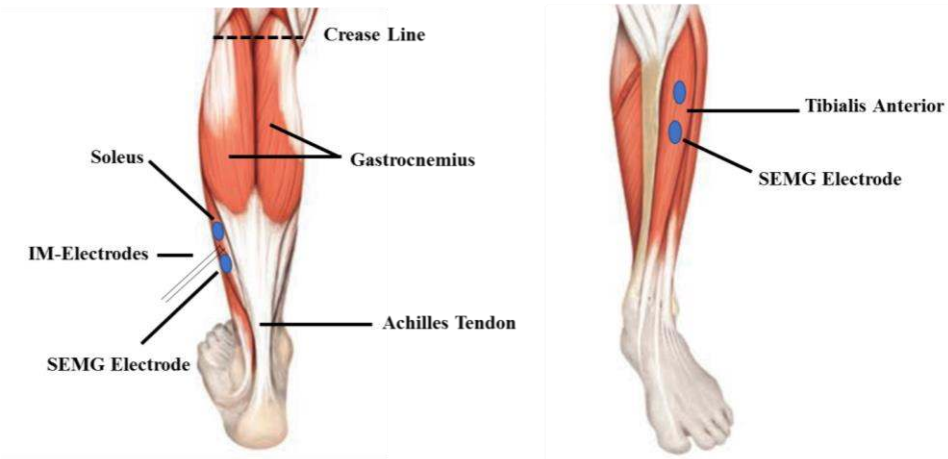


Figure 2.3. Recording electrode localization used in Renshaw inhibition experiments

Modified from Tortora and Derrickson (2018).

During the experiments, the subjects lay on a bed comfortably in the prone position. Their right foot was fixed firmly to a constant panel with hook and look tapes around the sole. Constant current stimulator's anode (10 x 12 cm) was placed immediately

proximal to the patella, and a cathode (3 x 3 cm) was located on a slightly lateral part of the popliteal fossa (Ozyurt, Shabsog, Dursun, & Turker, 2018). The anode and cathode were fixed with a tight knee pad to avoid any movement. The electrical stimulus is delivered from the tibial nerve with a 1-2 s interstimulus interval and a 1 ms pulse width. SEMG recordings from TA muscle were monitored to investigate if there is any crosstalk during the Stimulation. After detecting optimal stimulation point, the subjects were asked to perform three 3 MVCs with a 1-minute rest between them.

M-response threshold and maximum M-response (M_{max}) were obtained. M-response threshold was found by decreasing the stimulus intensity to get the smallest detectable M-response. For obtaining the M_{max} the stimulus intensity increased until no higher increment in the M-response amplitude was seen. The stimulus intensity used in the experiments is between 0 % M_{max} (just above the motor threshold) to 20 % Mmax. After all those protocols have completed, the subjects were asked to plantar flexion slightly to fire SMUs. The subjects monitored the SMU channel from a screen. With the detection of a stably firing SMU, the M-only Stimulation was initiated at predefined stimulus intensity and applied randomly. The average stimuli used is 476 ± 152 (mean ± SD). The rectified averaged SMU channel was monitored online through the experiments to control the inhibitory effect of stimulus. Also, the averaged SEMG channel was tracked online to make sure the stimulus did not evoke any H-reflex (the M response expected in 5-20 ms and H-reflex in 30-40 ms). The detailed analysis will be explained in the 2.3 Analysis section.

Masseter Inhibition Experiments

Five subjects participated in the masseter inhibition experiments (4 males, one female; aged 24-52 years). 2 of the subjects attended the experiments twice, and at least two different SMU recordings were performed in all experiments.

SEMG and IM-EMG recordings are made from the right masseter muscle. Bilateral SEMG electrodes were placed, one approximately 2 cm above mandibular angle and the other 4 cm above about temporomandibular level. The IM-EMG inserted between the SEMG electrodes as described in the 2.1 section (Fig 2.4). The ground electrode was located on the frontal bone.

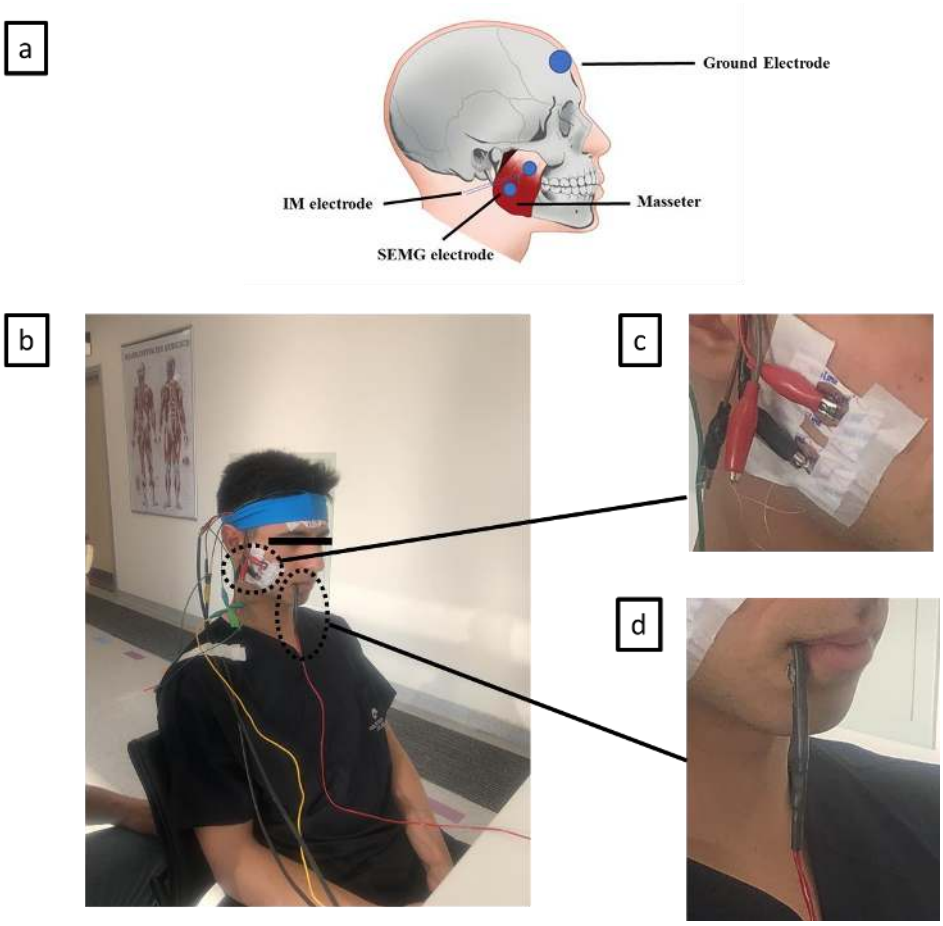


Figure 2.4. Electrode placement of masseter inhibitory and masseter stretch reflex experiments.

(a) Electrode localization, **(b)** Demonstration of the electrodes on a subject **(c)** Surface and IM-EMG electrodes magnified view **(d)** Stimulation electrode magnified view. Figure A was modified from Skármeta, Espinoza-Mellado, and PedroChana (2018).

The subjects were seated in an upright position in an armchair. The electrical stimulus is delivered by a special electrode produced and used by Türker and Miles in their earlier studies (Turker & Miles, 1985). The stimulation probe has a U shape holder, hanged to the right lower lip firmly. The cathode part of the probe was a two mm^2 diameter silver disc and clipped tightly to the oral mucosa. The anode of the probe stayed on the skin of the lip (3.7 mm^2 silver), and ultrasound gel was applied here to facilitate the current pass directly through the lip (Fig 2.4 D). The small dental cotton was placed between the upper and lower jaws (to the back part of the mouth on grinders) bilaterally to avoid jaw hitting.

The stimulation width was 0.05 ms square-wave. The stimulation intensity was eight times the sensory threshold (Uginčius, Yilmaz, Sebik, & Türker, 2017) delivered in 1-3 seconds interstimulus intervals randomly to avoid reflex habituation (Desmedt & Godaux, 1976). The sensory threshold (ST) was found by increasing the stimulus intensity starting from low amplitudes. The lowest intensity perceived is accepted as ST as described in CSP experiments above. Eight times ST chosen criteria were to create painful stimuli and reveal apparent inhibition (Uginčius et al., 2017). The number of stimulations delivered was 165 ± 36 (mean ± SD). Maximum voluntary contraction (MVC) was recorded three times; each lasted 5 seconds with 10 seconds of rest to find the contraction level during the experiment. Reaction time was also obtained to ensure that the subjects did not contaminate the natural inhibition with the expectation of painful stimuli. For the measurement of reaction time, the subjects were asked to bite their teeth when they perceive the stimulus. It was repeated at least three times for each subject.

After these protocols were completed, the subjects were asked to contract their masseter muscle very slightly to activate an identifiable SMU. Immediately after, the predetermined stimulus intensity was applied to examine if it generates an inhibition on the ongoing EMG activity. If inhibition was confirmed, the experiments have proceeded with decided stimulus intensity, and subjects follow their SMU activity from a feedback monitor. The analysis will be explained in the 2.3 section.

Excitatory Post Synaptic Potential (EPSP) Profile Estimation Experiments

Excitatory synaptic inputs cause an increase in spike occurrence probability and instantaneous discharge rate in tonically firing motor units. Using these probability and discharge rate increases can be a beneficial approach to estimate the excitatory postsynaptic potential profile (EPSP) (Powers & Türker, 2010). Thus, probability-based PSTH and discharge rate-based PSF methods were used together in this thesis with CUSUM to detect the excitation profile more efficiently.

Masseter Stretch Reflex

Eight subjects participated in the study, but only in two subjects in four experiments (2 males; aged 24 and 26 years), the excitation meets the expectation of this study. These two participants attended experiments twice, and multiple SMU recordings were

collected from them (8 SMU). Although the subject number was low, the main criteria of our statistics are the SMU number. Even so, this part of the thesis will be evaluated as preliminary results, and the subject number will be increased in the future.

The preparations and electrode positions were similar in all Masseter inhibitory experiments (Fig 2.4). The small dental cotton was placed between the upper and lower jaws bilaterally to avoid hitting the jaws since that can give rise to periodontal inhibition.

The subjects were seated in an upright position in an armchair. A reflex hammer was used to evoke stretch reflex in the masseter muscle. It was applied by tapping the mandible with a downward direction at the chin when the mouth was open slightly. Tap stimulus was applied in 1-3 seconds of interstimulus intervals. Three MVCs (each lasted 5 seconds with 10 seconds of rest) and reaction times were recorded before the experiments. Subjects were then asked to activate SMUs, and stimulus intensity was decided by monitoring the SMU channel around the stimulus time. The stretch reflex latency is known as a range of 6-10 milliseconds (Murray & Klineberg, 1984). Therefore, the optimal stimulus intensity was decided as three out of ten consecutive stimuli that the SMUs occur where stretch reflex was expected. Therefore, during the experiments, the stimulus intensity was tracked via real-time triggering of the SMU channel, and the window was updated simultaneously to avoid missing any trigger-related event. Stimulus intensities induced between 1 and 5 SMUs out of 10 (the time interval where masseter stretch reflex anticipated) was used. Optimum stimulus intensity was decided by this approach to reveal the detailed profile of EPSPs. The effective stimulus intensity was 27.25% ± 18.94 (mean ± SD). This intensity was calculated from PSTH analysis (will be explained in the 2.3 Analysis section).

The number of stimulations delivered was 348 ± 140 (mean ± SD). The analysis of the experiment will be explained in the 2.3 section.

Transcranial Magnetic Stimulation (TMS) Experiments

Nineteen subjects (10 males and 9 females; aged 18-30 years) participated in this experiment. Data collected in our previous TMS research (Özyurt et al., 2019) was used and reanalyzed for the investigation of EPSP in this thesis.

The experiments were performed on the right leg TA muscle (20 subjects) and right-hand abductor pollicis brevis (APB) muscle (4 subjects).

The SEMG electrodes were placed bilaterally on TA muscle 4 cm apart and APB muscle 2 cm apart since the muscle size is small. IM-EMG electrodes were inserted between SEMG electrodes as prescribed in detail in previous sections (2.1). A lip clip was used as a ground electrode for IM-EMG and SEMG recordings (Türker et al., 1988). 20000 Hz sampling rate was used for both IM-EMG and SEMG recordings. IM-EMG was filtered with a 200-10000 Hz, and SEMG was with a 20-10000 Hz bandpass filter.

The subjects were seated comfortably in a chair during the experients. Three MVCs were performed (each lasted 5 seconds with 60 seconds rests) before the experiments. Subjects were then performed dorsiflexion of their right foot to activate their TA muscle and fire SMUs. Optimal stimulation regions for transcranial magnetic stimulation were investigated when steadily firing, and recognizable SMUs were obtained. A double cone coil electrode (110 mm double cone coil) was used to stimulate the corresponding area of the left hemisphere for the right TA, and a figure of eight coil (D70 mm Alpha coil) was used for the right APB muscle. The motor cortex was used as a reference point (centre of the head) to detect the optimal stimulation area. It was found by using the midpoint of the nasioninion line. The stimulation point was located by delivering suprathreshold stimuli around the C3 region (according to the head's 10-20 system). The figure of eight coil was angled 45 degrees relative to the nasioninion line from the midpoint.

To find the stimulation point corresponds to TA, double cone coil placed approximately to the middle of nasioninion line and oriented slightly in alignment with C3 area. Stimulation of those areas generates motor evoked potentials (MEPs), and peak-to-peak values were measured to decide the optimal stimulation area.

Stimuli were applied between 4-6 seconds randomly. The stimulus intensity was denominated in the active motor threshold. The active motor threshold was described as the lowest intensity at 5 out of 10 consecutive stimuli to evoke MEP of at least 100 μV in peak-to-peak amplitude. This value is measured from the SEMG channel while the subjects contact their muscle slightly. Thus, 150 % of the active motor threshold was used in experiments

After determining the stimulus point (a pen marked the location), the subjects contract their muscle slightly (APB or TA) to fire SMUs. The single-pulse TMS was

initiated while SMUs were identifiable and discharging steadily. During the experiments, subjects tracked their SMUs from a feedback monitor.

The stimulus intensity was checked from a real-time triggering of the SMU channel throughout the experiments. The number of SMUs was counted around the time interval where MEPs were expected to occur. This window was updated simultaneously to avoid missing any trigger-related event. Stimulus intensities evoke between 10 and 50 SMUs out of 100 (the time interval where MEPs anticipated) was accepted. This approach was thought to be an efficient method to estimate the EPSP profile. The stimulus intensity was measured at an average strength of 33.65 ± 1.37 (mean ± SEM) for TA and 38.13%.± 1.17 (mean ± SEM) for APB. The number of stimuli applied to the motor cortex was 541 ± 19 (mean ± SD) for TA and 320 ± 32 (mean ± SD) for APB.

H-Reflex Experiments

Six participants (4 males, 2 females, aged 18-27 years) were attended this experiment. One subject participated in the experiment twice.

SEMG recordings were made from the right soleus muscle and placed 4 cm apart after preparations have completed. IM-EMG was inserted between SEMG electrodes, as explained in detail in the 2.1 section. A lip clip was used as a common ground for SEMG and IM-EMG electrodes (Türker et al., 1988).

The subjects were laid comfortably in the prone position on the examination bed. Then, they were asked to perform plantar flexion against a fixed panel with their right foot. Constant current stimulator's anode (10 x 12 cm) was placed proximal to the patella. The horizontal distance between the condyles was measured, and the cathode was placed at the midpoint of the popliteal fossa to elicit the H-reflex response on the soleus muscle (Ozyurt et al., 2018). Hmax and Mmax values were obtained in the relaxed position. Three MVCs were recorded before the experiments; each lasted 5 seconds with 10 seconds of rest. The electrical stimulus was delivered with a width of 1 msec (as square pulses) and in 2-3 seconds of interstimulus intervals randomly.

The subjects performed plantar flexions to activate their soleus muscle. The SMUs were recruited with this slight contraction. They were asked to fire their SMUs consistently while tracking their SMU activity from a feedback monitor. The electrical stimulus was initiated when only one SMU was discharging (a few concurrently firing

SMUs were also excepted if they were separable). The optimal stimulus intensity was determined as three out of ten consecutive stimuli that the SMUs occur where H-reflex was expected to occur, 30-45 msec (Miles et al., 1989). The stimulus intensity was tracked via real-time triggering of the SMU channel during the experiments. Stimulus intensities induced between 1 and 5 SMUs out of 10 (the time interval where H-reflex was anticipated) were used. The effective stimulus intensity was 16.11% ± 4.9 (mean ± SD). It was calculated from PSTH analysis.

The number of stimulations delivered was 315 ± 131 (mean ± SD). The analysis of the experiment will be explained in the 2.3 section.

2.2.2. Rat Brain Slice Recordings

Data used in this part of the thesis was procured from Turker and Powers (1999). They have investigated the effects of large excitatory and inhibitory stimulus on motoneuron discharge rate and probability directly by using rat hypoglossal motoneuron recordings.

In their experiments, 18 to 24 days old Sprague-Dawley rats were used. Experiments were performed on 400 μm thick brain slices derived from rat hypoglossal motoneurons. The surgical protocols were explained in detail in the publication (Turker & Powers, 1999). Sharp microelectrodes were inserted into hypoglossal motoneuron soma to record the membrane potential and inject current waveforms with background noise. Current waveforms were injected with superimposed noise to induce repetitive discharge to simulate the synaptic drive that usually occurs in the physiological activation of motoneurons in humans. Forty-two second-long injected current waveforms stimulated these repetitive discharges, which consist of four constituent: a 35-second suprathreshold step, a 26.2-second zero-mean random noise waveform that starts five seconds after step initiation, injected current transient train lasts 26.2 seconds (begins with the noise waveform), two series of eight 1 ms, 1 nA hyperpolarizing current pulses administered before and after the current step (Turker & Powers, 1999, 2005). The noise waveforms superimposed on the current steps in the ranges used in this study stimulate similar variabilities and discharge rates detected from voluntarily active human motoneurons. In addition, an extra current transient train was added to the injected current waveform to mimic the repetitive en masse stimulation effects on afferent fibres (PSPs). These current transients were designed to simulate the synaptic

currents that give rise to EPSP or IPSPs with their wide range of amplitude (0.1– 0.5 nA) and time course (0.1–20 ms rise time, 1–50 ms half-width). Current transient intervals were distributed randomly between 200 ms and 600 ms. Discharge frequencies and spike times were used to construct PSFs and PSTHs, respectively. PSTHs and PSFs were built by utilizing three to six repetitions of the injected current waveform (each contained 66 current transients). Additionally, PSTH-CUSUMs and PSF-CUSUMs were constructed (Fig 2.6).

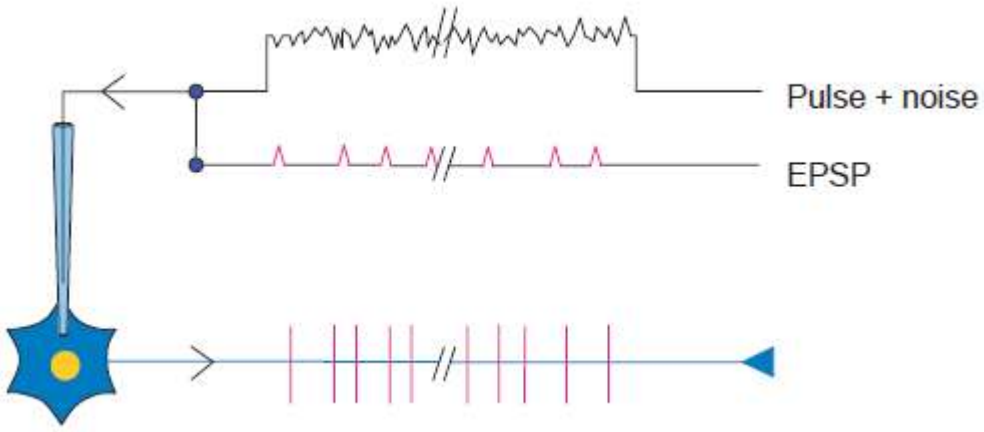
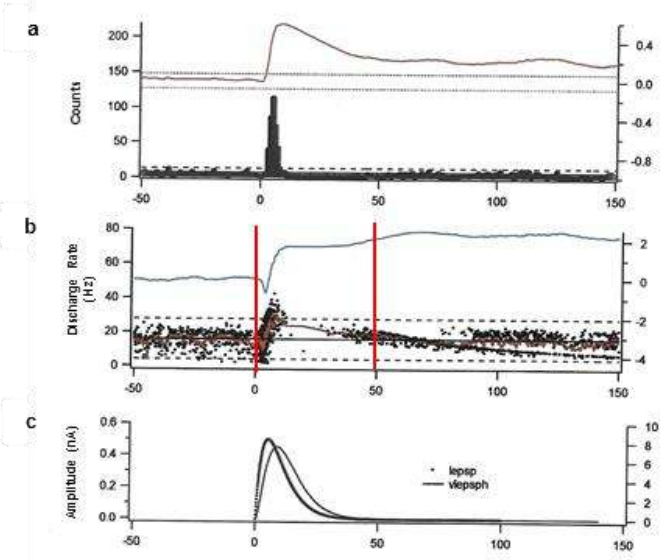


Figure 2.5. Production of stimulus induced PSPs in rat hypoglossal neurons.

Current pulses with superimposed noise were injected to induce repetitive discharge. Thereby synaptic drive during physiological activation was simulated. An extra current transient train was added to the injected current waveform to mimic the repetitive en masse stimulation effects on afferent fibers (EPSPs). The figure was taken from Turker and Powers (2005).



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0	pk area	0.591814	mtn13
1	pk dur	9	mtn14
2	mean counts	7.27363	mtn15
3	sd counts	2.85127	mtn16
4	pk av freq	30.2292	mtn17
5	ttpk av freq	9.1	mtn18
6	dur freq chng	63.2	mtn19
7	mn bckgr freq	15.8492	mtn20
8	sd bckgr freq	5.90404	mtn21
9	nresp	3036	
10	cusum pre rng	0.0376652	
11	cusum 2nd min	0	
12	time to min	0	
13	cusum 2nd max	0	
14	time to max	0	

Figure 2.6. The output obtained from EPSP injection to rat hypoglossal neuron.

An EPSP (in c) was injected into a steadily discharging hypoglossal motoneuron. The response of the neuron was evaluated with **(a)** PSTH (peristimulus time histogram) **(b)** PSF (peristimulus frequencygram), duration of excitation was measured from here (the red vertical lines) **(c)** Injected EPSP current (with 50 ms duration), **(d)** The table demonstrates injected EPSP current information and PSF results. Motoneuron background frequency values were taken from this table (marked in red box). The red line at the top of a and the dark blue line in b is the CUSUM of PSTH and PSF, respectively.

Motoneuron background firing frequency and duration change data (Fig 2.6 D) were used in this thesis to construct a linear graph which will be explained in detail in the Results chapter.

2.3. Analysis

The shape of the single motor unit action potential was identified as a template in Spike2 program. SMUs whose shape fit this preestablished template created acceptance pulses (as instantaneous frequency) (Fig 2.7). The interval histograms were made to affirm the distribution of SMU interspike intervals. The confirmed acceptance pulses were used to construct peristimulus frequencygrams (PSF) and peristimulus time histograms (PSTHs) around stimulation time. PSTH histogram demonstrates the timing of spikes and superimposes them against the stimulus time; PSF is the superimposition of instantaneous discharge rates of an SMU around the stimulus time. 600 ms time window (300 ms prestimulus and 300 ms poststimulus) and 0.1 ms binwidth were chosen to build PSTHs and PSFs.

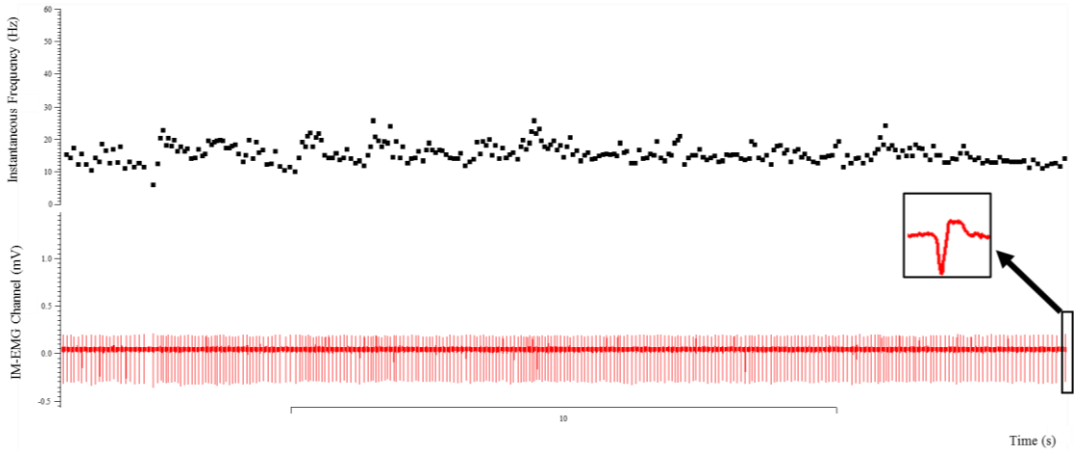

Figure 2.7. Sample IM-EMG recording and its acceptance pulses.

The bottom figure was a part of a raw IM-EMG recording showing SMUs. These SMUs were converted to acceptance pulses on their shapes with template matching Spike 2 (upper figure).

Synaptic noise causes fluctuations in both PSF and PSTH analysis; therefore, the CUSUM-error box (Brinkworth & Turker, 2003; Turker, Yang, & Brodin, 1997) was constructed from PSF and PSTH to reveal the subtle but perpetual changes. The prestimulus average bin value was found (Number of events for PSTH and discharge rate for PSF) and subtracted from each bin value in the entire analysis period (-300 to

+300 ms period). After that, the residual values left in each bin were summed to procure the CUSUM (Ellaway, 1978). If poststimulus residual values were higher than the average, then CUSUM increases; if it is lower than the average, CUSUM decreases. The maximum deflection in the prestimulus was obtained, and a symmetrical error box was created from the maximal prestimulus deflection. A significant event was determined if the poststimulus CUSUM deviation was greater than the error box limits (Brinkworth & Turker, 2003). If those deflections going downwards, they were categorized as inhibition (the red lines are CUSUMs and the decrease initiates with the green line shows the IPSP latency in Fig 2.8); if going upwards, they classified as excitation (the increase starts with the green line shows the EPSP latency in Fig 2.9).

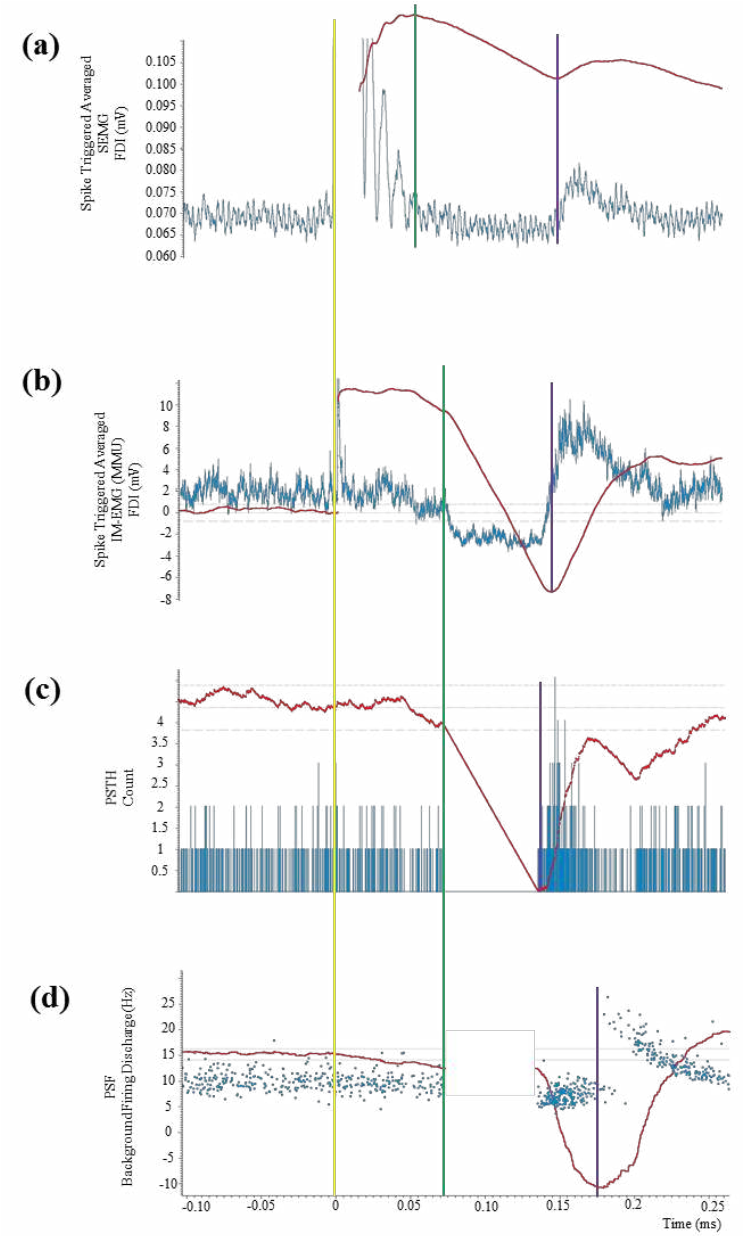

Figure 2.8. Data analyzed from a cutaneous silent period (CSP) experiment to estimate the IPSP profile.

(a) Spike-triggered average analysis of first dorsal interosseous muscle (FDI) SEMG recording. **(b)** FDI muscle IM-EMG recording analysis with Spike-triggered average method. **(c)** PSTH and **(d)** PSF analysis constructed from SMU analysis. The yellow line shows the electrical stimulus time point. Red lines are the CUSUM of each analysis, and the horizontal dashed lines are CUSUM-Error Boxes. Green lines demonstrate the latency of the inhibition, and purple lines are the end of the inhibition. IPSP duration is the time between the purple and green lines. The number of stimuli delivered in this experiment was 274, and the background firing rate was 10.25 ± 1.76 Hz.

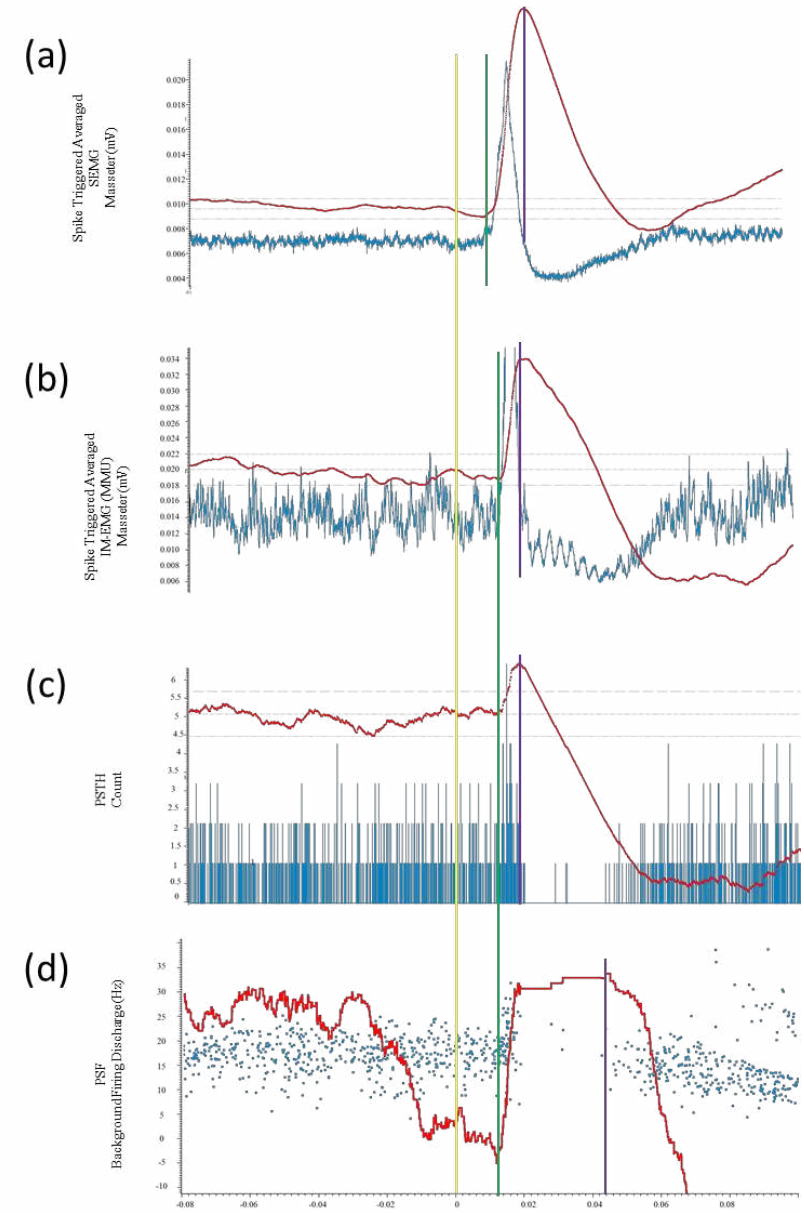

Figure 2.9. Data analyzed from a masseter stretch reflex experiment to estimate EPSP profile.

(a) Spike-triggered average analysis of masseter muscle SEMG recording. **(b)** Masseter muscle IM-EMG recording analysis with Spike-triggered average method. **(c)** PSTH and **(d)** PSF analysis constructed from SMU analysis. The yellow line shows the electrical stimulus time point. Red lines are the CUSUM of each analysis, and the horizontal dashed lines are CUSUM-Error Boxes. Green lines demonstrate the latency of the excitation, and purple lines are the end of the excitation. EPSP duration is the time between the purple and green lines. The number of stimuli delivered in this experiment was 410, and the background firing rate was 15.47 ± 2.91 Hz.

PSTHs are considered efficient analysis tools at showing the PSP latency correctly, and PSFs are shown better to obtain the profile of the PSP (Miles, 1997; Turker & Powers, 2003, 2005). For this reason, PSTHs were used to acquire the latency, and PSFs were to end of the duration of PSPs. The subtraction of these two values presents the PSP duration. The background discharge rate of the SMUs was calculated from PSF analysis. The prestimulus discharge rates between -200 ms to -10 ms were procured and averaged. The relation between the background firing frequency of SMUs and the duration of PSPs was investigated by linear regression. The extrapolation approach was applied to this linear regression analysis to find the estimated duration of synaptic potentials when no motor neurons were discharging (Ozyurt et al., 2019). "0 Hz" background firing rate was placed in linear regression formula to estimate the silent motoneuron duration.

The corresponding data (background discharge rate and duration) to human experiments were procured from rat brain slice data. The linear regression analysis was also performed on this data, similar to human analysis. Here the duration of the given EPSP is known, which is 50 ms. The estimation of genuine motoneuron firing frequency can be found by placing this value on linear regression analysis of slice recordings. Since this background firing frequency is acquired from direct recordings, it is thought to be a more accurate value than indirect results obtained from human recordings. Therefore this value was used in human linear regression analysis to find the corresponding synaptic potential duration. It is hypothesized that this would be a useful approach to estimate the real duration of PSPs.

Although analysis from SMUs were contribute mostly to main aim of this thesis SEMG and multi motor unit (MMU) analysis were also performed for comparison. Rectified and DC removed SMU recording was used as a source for MMU analysis, and spike-triggered averaging method (-300 to +300 ms) was applied to this data. The same approach used on DC removed and rectified SEMG recordings. CUSUM charts were also built from SEMG and MMU data for more precise results (Fig 2.8 and 2.9).

The effective stimulus was calculated in EPSP experiments from PSTH-CUSUM analysis. The first deflection of CUSUM higher than the prestimulus value was obtained. The amplitude of this deflection was measured with two horizontal cursors. This value was divided by stimulus number and the percentage of the effective stimulus

was measured by multiplying the value by a hundred. The effective stimulus was used to investigate the relationship between stimulus intensity and duration.

GraphPad Prism 8.0.1 was used for linear regression analysis between motoneuron background firing frequency and duration. PSP duration obtained from PSF-CUSUM, PSTH-CUSUM, SEMG-CUSUM, and MMU-CUSUM was compared with Kruskal-Wallis using Dunn's for multiple comparisons.

PSP latency obtained from PSTH-CUSUM, SEMG-CUSUM, and MMU-CUSUM was also compared with Kruskal-Wallis using Dunn's for multiple comparisons. PSTH- and MMU-CUSUM was compared with Mann-Whitney Test. Shapiro-Wilk, D'Agostino & Pearson, Kolmogorov-Smirnov, and Anderson-Darling normality tests were applied to check normality before Kruskal-Wallis.The significance level used was $p<0.05$.

CHAPTER 3
RESULTS

3.1. Rat Brain Slice Recordings

Data used in this part of the thesis was acquired from Turker and Powers (1999). Duration and background firing frequency of the rat hypoglossal neurons were used for linear regression analysis. Duration of the PSPs was calculated from PSF-CUSUM analysis. Since the injected PSP duration was predetermined in rat hypoglossal neuron experiments, the motoneuron background discharge rate that delivers the exact PSP duration was obtained using a correlation plot between the discharge rate and PSP duration.

3.1.1. Inhibitory Postsynaptic Potential Injection to Rat Hypoglossal Neurons

Data obtained from 8 experiments were used to build the linear graph. The mean duration gained from these experiments was 34.08 ± 6.85 ms, and the mean background firing frequency was found 11.30 ± 2.82 Hz.

The background firing frequency of the motor units has been known to affect the inhibition duration. Linear regression was performed on these eight experiments; the duration of these units procured information about the time course of the inhibition (Fig 3.1). The units firing with a low background discharge rate were inhibited significantly more than high-frequency units ($p= 0.022$). The estimation duration of these motor units was found with the **extrapolation approach** (Ozyurt et al., 2019). According to this method, it has been found that if all the motor units were silent (0 Hz motor unit firing frequency), the inhibition duration would be 55.46 ms. This value was calculated from the linear regression formula.

More importantly, the main aim is to find the motoneuron background discharge rate that gives the exact IPSP duration. The IPSP duration injected was 50 ms; this value was used in the regression formula to find the background discharge rate of 2.88 Hz (Fig 3.1).

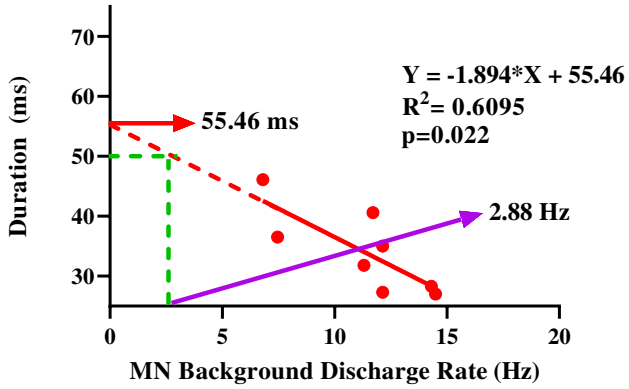

Figure 3.1. The relationship between the duration of the inhibition and background discharge rate (n=8).

3.1.2. Excitatory Postsynaptic Potential Injection to Rat Hypoglossal Neurons

Fourteen experiments were used to build the linear graph. The mean duration obtained from EPSP injection experiments was 23.93 ± 13.65 ms, and the mean background discharge rate was found 12.04 ± 4.10 Hz.

The linear regression showed that the background discharge rate significantly affects the excitation duration (p = 0.0049). There is a positive correlation between the motoneuron discharge rate and duration of the excitation (Fig 3.2).

Injected EPSP duration was 50 ms in these experiments; using this value in linear regression formula, 23.50 Hz motoneuron discharge frequency was acquired (Fig 3.2)

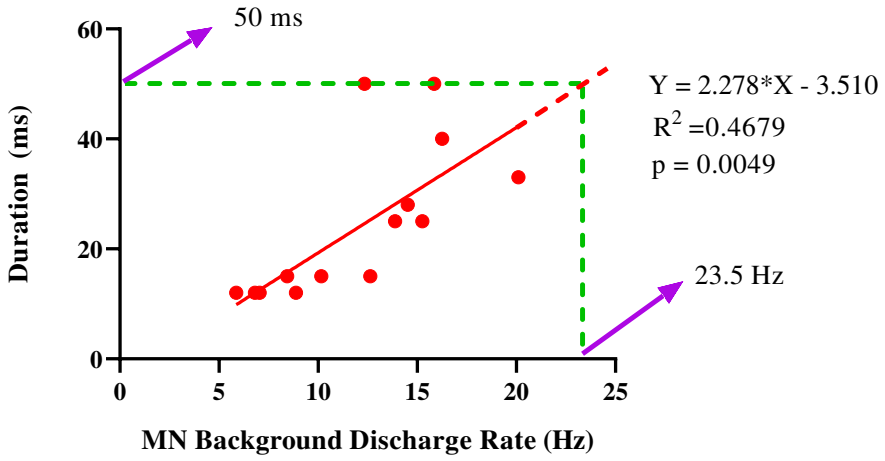

Figure 3.2. The relationship between the duration of the excitation and background discharge rate (n=14).

3.2. Human Experiments

Both SMU and SEMG recordings were made in human experiments. SMU results were evaluated with PSTH-CUSUM and PSF-CUSUM methods, MMU and SEMG results were analyzed by the spike-triggered averaging (STA) method. Linear regression analysis was performed to investigate the relationship between PSP duration and background firing frequency. The extrapolation approach was used to estimate the PSP duration when all MNs were silent. Data from rat hypoglossal motoneurons were combined with human recordings to determine the exact duration of the PSPs.

3.2.1. Inhibitory Postsynaptic Potential (IPSP) Profile Estimation

The duration and latency and of the inhibition were calculated from PSF-CUSUM and PSTH-CUSUM, respectively. Discharge rate versus PSP duration correlation plots was constructed similar to rat brain slice analysis (Section 3.1.1). The motoneuron discharge rate data obtained from these slice experiments were used in human IPSP linear regression analysis to estimate the actual duration of the IPSP. MMU and SEMG were used to procure latency and duration information of the IPSPs to compare PSF-CUSUM and PSTH-CUSUM analysis.

Cutaneous Silent Period (CSP) Experiments

20 single motor units from 9 subjects were recorded from FDI muscle; for each SMU, duration, and latency were calculated with PSF-CUSUM and PSTH-CUSUM, respectively (Fig 3.3). The duration was calculated by PSTH-CUSUM only for comparison.

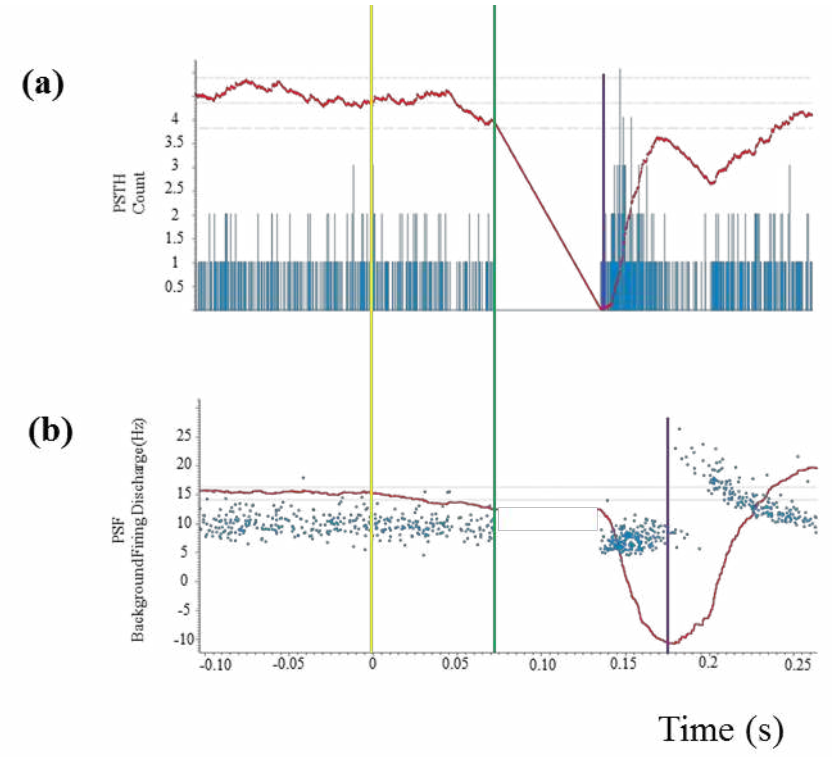

Figure 3.3. Data analyzed from a cutaneous silent period (CSP) experiment to estimate IPSP characteristics with PSTH-CUSUM and PSF-CUSUM methods. PSTH-CUSUM was used to obtain latency and PSF-CUSUM to the duration of the inhibition. Duration of the inhibition was found to be shorter with PSTH-CUSUM than PSF-CUSUM analysis (64.04 ms and 107.12 ms, respectively). The yellow line shows the electrical stimulus time point. Red lines are the CUSUM of each analysis, and the horizontal dashed lines are CUSUM-Error Boxes. Green lines demonstrate the latency of the inhibition, and purple lines are the end of the inhibition. IPSP duration is the time between the purple and green lines. The number of stimuli delivered in this experiment was 274, and the background discharge rate was 10.25 ± 1.76 Hz.

Additionally, MMU analysis was performed for comparison. The mean ± SD duration of CSP was 136.49 ± 29.31 ms with PSF-CUSUM, 74.94 ± 19.34 ms with PSTH-CUSUM, and 73.34 ± 16.67 with MMU-CUSUM analysis. Thus, the duration of the CSP inhibition was obtained significantly higher with PSF-CUSUM than the two other analysis methods ($p<0.0001$). (Fig 3.4a).

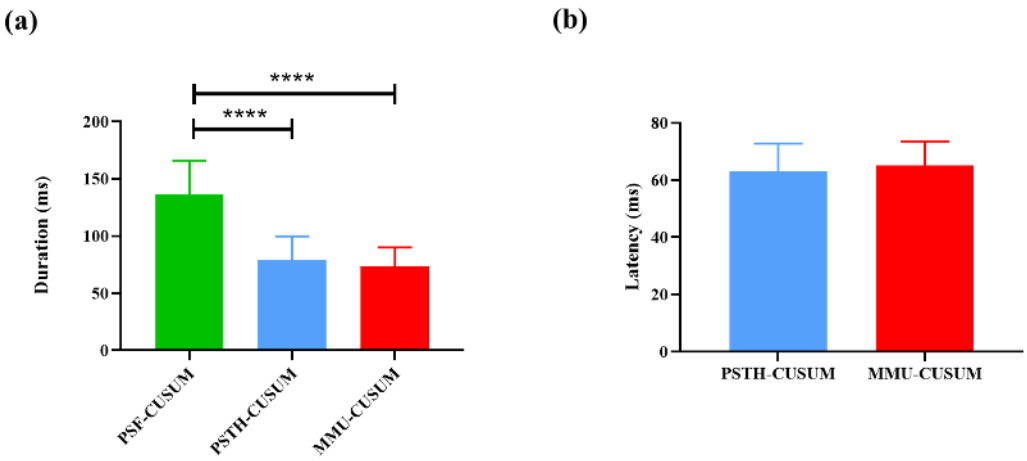

Figure 3.4. Characteristics of CSP inhibition.

(a) Duration (b) latency of the CSP inhibition analyzed by different analysis methods. n = 20 Error bars are standard deviations and ****p<0.0001.

The mean ± SD latency of CSP was 62.95 ± 9.91 ms with PSTH-CUSUM, 65.04 ± 8.53 with MMU-CUSUM. No significant difference was found between PSTH- and MMU-CUSUM (p = 0.4987) (Fig 3.4b).

SEMG recording analysis was not shown since it failed to detect latency and duration of the inhibition. Due to broad stimulus artifact, the presentation of inhibition characteristics was hindered. The proximity of the stimulation area (from the 5th finger) and recording electrodes (from FDI muscle) causes these extended stimulus artifacts.

Linear regression analysis was performed to assess the relationship between the duration of CSP and background motoneuron discharge rate (similar to data analysis obtained from rat hypoglossal neurons in section 3.1.1). Duration of the CSP was found from PSF-CUSUM (136.49 ± 29.31, mean ± SD), ms and motoneuron background discharge rate was from prestimulus part of the PSF analysis (-200 to -10 ms, 8.90 ± 2.43 Hz, mean ± SD).

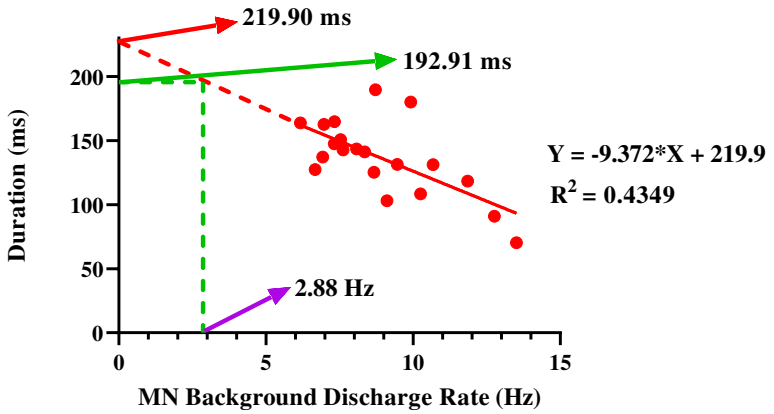

Figure 3.5. The relationship between the duration of the CSP and background discharge rate (n=20).

The dashed red line was extrapolated linear regression line to calculate the duration of silent MNs; the dashed green line extended to the y-axis represents the estimated duration of CSP calculated using the background discharge rate obtained from rat hypoglossal neuron experiments (2.88 Hz) in CSP linear regression analysis.

The linear regression analysis revealed the significant relationship between CSP duration and motoneuron background firing frequency ($p = 0.0016$). Motoneurons that fire at a slower background rate was found to be inhibited significantly more than the faster ones. The estimation of the CSP duration when the motor units were silent (0 Hz) was found 192.91 ms from the extrapolation approach (Fig 3.5).

The background discharge rate, which provides the predetermined IPSP duration (50 ms), was calculated from rat hypoglossal motoneurons, and 2.88 Hz was found from this calculation (Section 3.1.1). We hypothesized that if we implant this value to the linear regression analysis procured from human CSP experiments, the genuine duration of the CSP can be estimated. Thus, 192.91 ms was found by putting 2.88 Hz value obtained from rat slice recordings into linear regression formula ($Y = -9.372X + 219.90$), which was calculated from human CSP experiments (Fig 3.5)

Renshaw Inhibition (RI) Experiments

IM-EMG and SEMG recordings were made from soleus muscle in the course of tibial nerve stimulation. Twenty-nine units were collected from 10 subjects. In addition, PSF-CUSUM and PSTH-CUSUM charts were constructed from SMUs to procure the duration and latency of Renshaw inhibition, respectively (Fig 3.6).

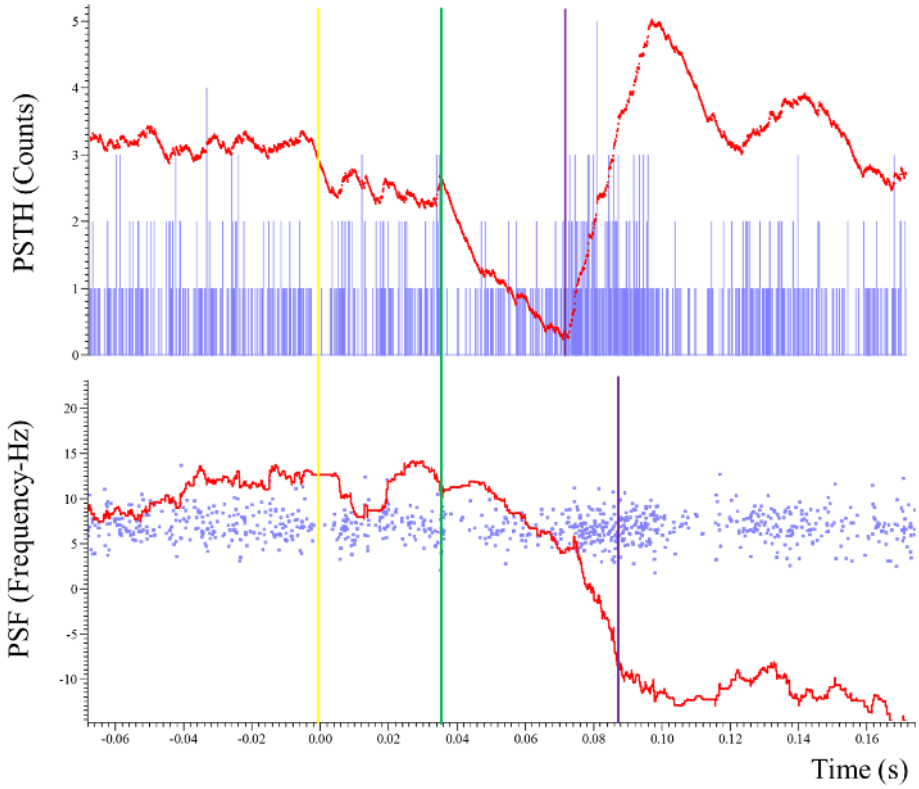

Figure 3.6. Sample data analyzed from Renshaw Inhibition (RI) experiment to estimate IPSP characteristics with PSTH-CUSUM and PSF-CUSUM methods. PSTH-CUSUM was used to obtain latency and PSF-CUSUM to the duration of the inhibition. Duration obtained with PSTH-CUSUM was shorter than PSF-CUSUM (36 ms and 52 ms, respectively). The yellow line shows the electrical stimulus time point. Red lines are the CUSUM of each analysis, green demonstrate the latency of the inhibition, and purple lines are the end of the inhibition. IPSP duration is the time between the purple and green lines. The number of stimuli delivered in this experiment was 612, and the background firing frequency was 6.9 Hz.

Duration of the RI was found 31.80 ± 5.58 ms with PSF-CUSUM, 14.14 ± 2.89 ms with PSTH-CUSUM, and 14.74 ± 3.20 ms with MMU-CUSUM (mean ± SD). Inhibition duration was found significantly longer with PSF-CUSUM analysis compared to PSTH- and MMU-CUSUM ($p<0.0001$) (Fig 3.7a).

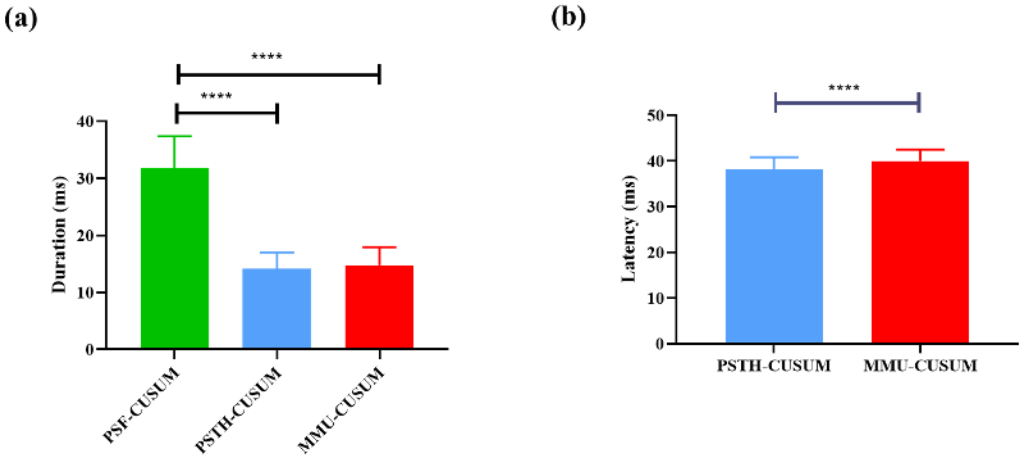

Figure 3.7. Evaluation of RI duration and latency with different analysis tools. n=29, error bars are standard deviations and ****p<0.0001.

The mean ± SD latency of RI was 38.17 ± 2.69 ms with PSTH-CUSUM, 40.04 ± 2.45 with MMU-CUSUM. Latency of the inhibition was found significantly lower with PSF-CUSUM analysis ($p<0.0001$)(Fig 3.7b).

SEMG recording analysis was failed to show latency and duration of the inhibition clearly. The presentation of the inhibition was evanished since the stimulus artifact was too broad.

Linear regression analysis revealed a significant negative relationship between the duration of RI and background discharge firing frequency ($p = 0.0076$). Duration of the RI was obtained from PSF-CUSUM (31.80 ± 5.58 ms, mean ± SD), ms and motoneuron background firing frequency was from the prestimulus part of the PSF analysis (6.52 ±0.81 Hz, mean ± SD). RI duration was found 53.55 ms with extrapolation approach in silent motoneurons; that was shown in our previous study (Ozyurt et al., 2019).

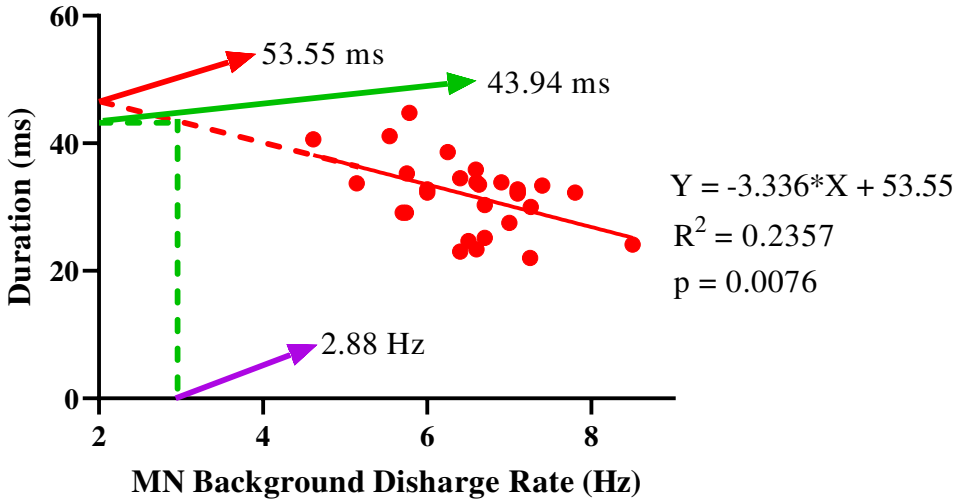

Figure 3.8. Duration of RI and background discharge rate relationship (n=29). The dashed red line is extrapolated linear regression line to calculate the duration of silent MNs; the dashed green line extended to the y-axis represents the estimated duration of RI calculated using the background discharge rate obtained from rat hypoglossal neuron experiments (2.88 Hz) in RI linear regression analysis.

The background discharge rate acquired from rat slice recordings (that provides the predetermined IPSP duration, 50 ms) was used to estimate actual RI duration. As a result, 43.94 ms was found by inserting this value (2.88 Hz) into the linear regression formula (Y = -3.336X + 53.55) obtained from RI experiments (Fig 3.8).

Masseter Inhibition Experiments

Fourteen single motor units from 5 subjects (2 subjects participated twice) were recorded from masseter muscle during high-intensity electrical stimulation; each SMU, duration, and latency were analyzed with PSF-CUSUM and PSTH-CUSUM, respectively. Short and long-latency inhibitions were seen together in some of the experiments; these inhibitions were calculated as a compound inhibition (Fig 3.9).

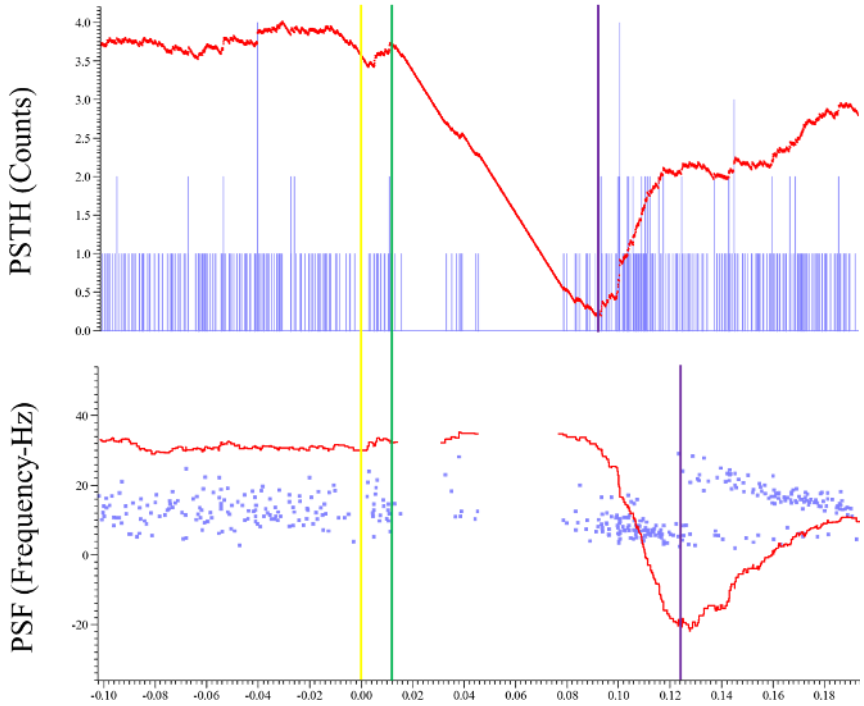

Figure 3.9. Analysis of masseter inhibition with PSTH-CUSUM and PSF-CUSUM methods obtained from a subject used in these experiments.

PSTH-CUSUM was used to procure the latency and PSF-CUSUM to the duration of the inhibition. Duration of inhibition was shorter with PSTH-CUSUM than PSF-CUSUM analysis (78.3 ms and 11.54 ms, respectively). The yellow line shows the electrical stimulus time point. Red lines are the CUSUM of each analysis, green is the latency of the inhibition, and purple lines are the end of the inhibition. IPSP duration is the time between the purple and green lines. The number of stimuli delivered in this experiment was 146, and the background firing frequency was 12.99 ± 4.33 Hz.

111.61 ± 26.31 ms masseter inhibition duration was measured with PSF-CUSUM, 68.23 ± 17.52 with PSTH-CUSUM, and 70.75 ± 13.35 ms with MMU-CUSUM (mean ± SD). Inhibition duration PSF-CUSUM was detected significantly longer than PSTH- and MMU-CUSUM ($p = 0.0003$ and $p = 0.0009$, respectively) (Fig 3.10a).

The latency of the inhibition was 12.38 ± 1.62 ms with PSTH-CUSUM, 14.16 ±1.90 with MMU-CUSUM analysis. Latency of the inhibition was found significantly earlier with PSTH-CUSUM ($p = 0.0087$) (Fig 3.10b).

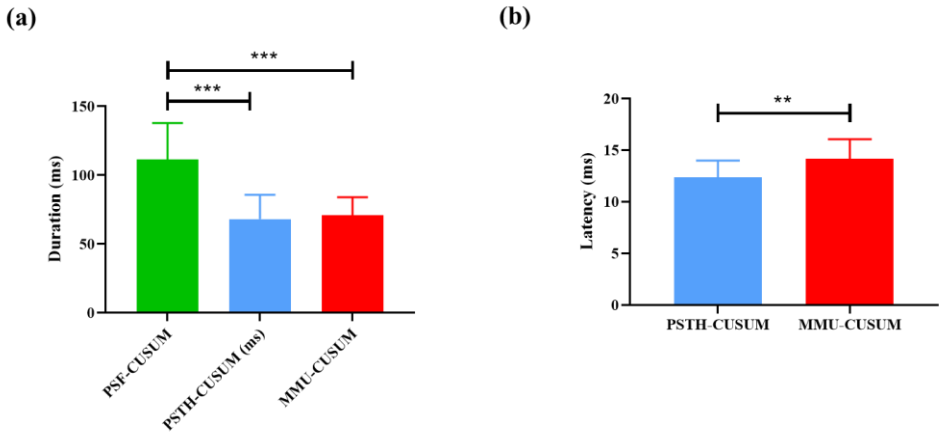

Figure 3.10. Evaluation of masseter inhibition (a) duration and (b) latency with various analysis methods.

n=14, error bars are standard deviations, ****p=0.0003 and p=0.0009, respectively in duration , **p = 0.0087 in latency analysis.

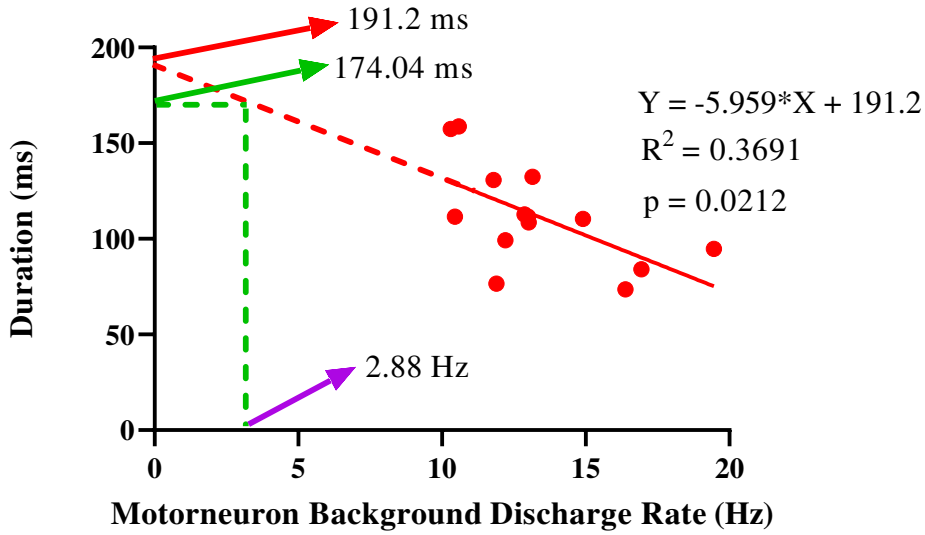

Figure 3.11. Masseter inhibition duration and background discharge rate relationship (n=14).

The dashed red line is extrapolated linear regression line to calculate the duration of silent MNs; the dashed green line extended to the y-axis represents the estimated duration of masseter inhibition calculated using the background discharge rate obtained from rat hypoglossal neuron experiments (2.88 Hz).

A significant opposite relationship was observed between the duration of masseter inhibition and background discharge rate ($p = 0.0212$). Masseter inhibition duration was 111.61 ± 26.31 ms and motoneuron background firing frequency 13.35 ± 3.84 Hz. Silent motoneuron duration for the IPSP was 191.20 ms via the extrapolation method. Masseter duration estimation was found at 174.04 ms using background discharge rate value obtained from rat slice experiments (2.88 Hz) in linear regression analysis.

3.2.2. Excitatory Postsynaptic Potential (EPSP) Profile Estimation

Excitation duration and latency were calculated from PSF-CUSUM and PSTH-CUSUM, respectively. EPSP duration and background discharge rate relationship was investigated with linear regression similar to rat brain slice analysis (Section 3.1.1). The motoneuron discharge rate data obtained from these slice experiments were used in human EPSP linear regression analysis to estimate the realistic duration of the EPSP. MMU and SEMG analysis were used for the comparison of PSF-CUSUM and PSTH-CUSUM analysis.

Masseter Stretch Reflex

Eight single motor units from two subjects (in four experiments) were recorded from masseter muscle during tap stimulation to the chin. Since the subject number is too low, these experiments will be continued, and these results are evaluated as preliminary. SMU duration and latency were analyzed using PSF-CUSUM and PSTH-CUSUM, respectively (Figure 3.12).

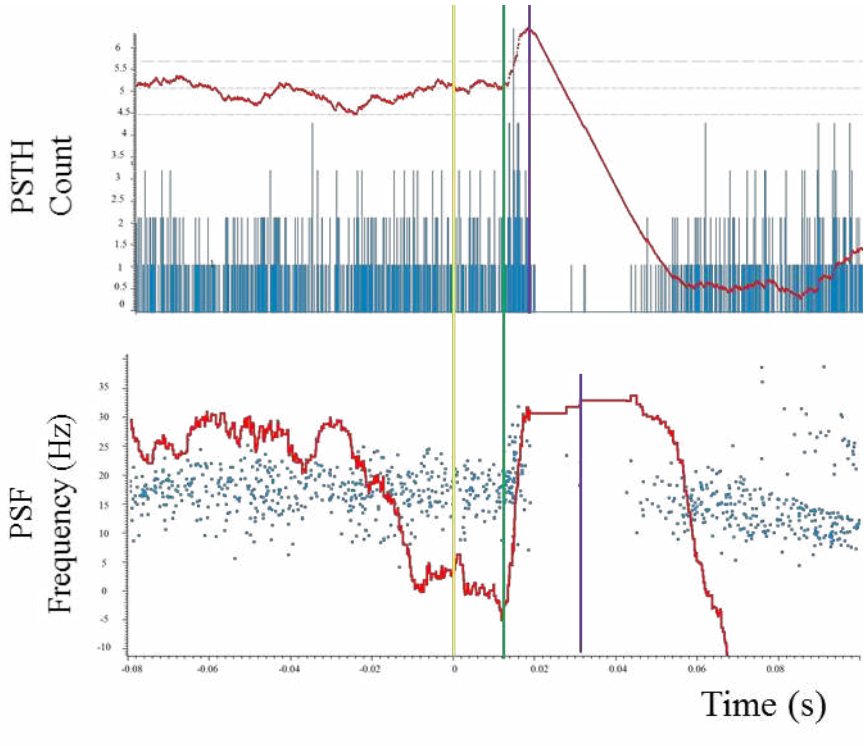

Figure 3.12. Data analyzed from a masseter stretch reflex experiment to estimate EPSP profile.

PSTH and PSF analysis constructed from SMU analysis. The yellow line shows the electrical stimulus time point. Red lines are the CUSUM of each analysis, and the horizontal dashed lines are CUSUM-Error Boxes. Green lines demonstrate the latency of the excitation, and purple lines the end of the excitation. EPSP duration is the time interval between the purple and green lines. The number of stimuli delivered in this experiment was 410, and the background firing frequency was 15.47 ± 2.91 Hz.

Masseter stretch reflex duration was calculated 9.70 ± 4.20 ms with PSF-CUSUM, 6.28 ± 1.85 ms with PSTH-CUSUM, and 8.45 ± 1.86 ms with MMU-CUSUM (mean ± SD). No significant difference was found between the groups ($p = 0.0625$) (Fig 3.13a).

The latency was found 11.25 ± 0.96 ms with PSTH-CUSUM and 11.86 ± 0.83 with MMU-CUSUM analysis. There is no significant difference between the groups ($p = 0.1510$) (Fig 3.13b).

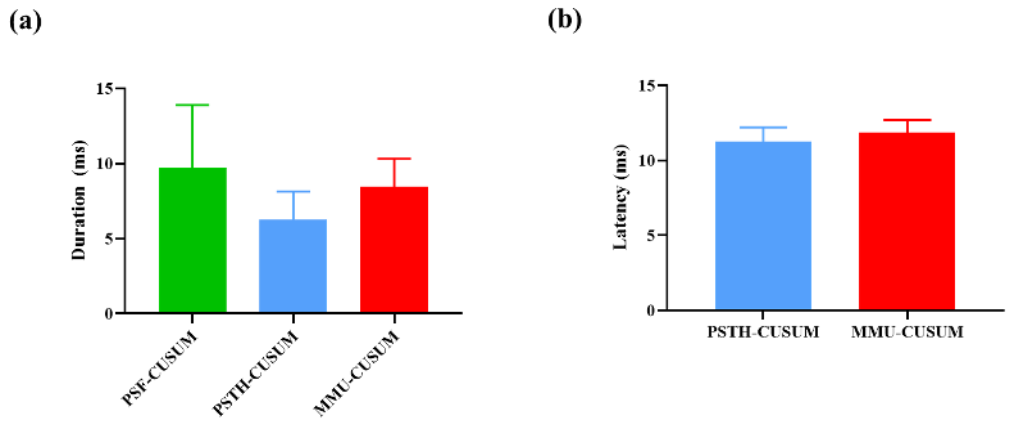

Figure 3.13. Masseter stretch reflex (a)duration and (b) latency measurements with various analysis methods

n=8, and error bars are standard deviations.

No significant relationship was observed between the duration of masseter inhibition and background discharge rate with linear regression analysis (p = 0.8263) (Figure 3.12). Mean masseter stretch reflex duration was detected 9.70 ± 4.20 ms with PSF-CUSUM, and motoneuron background discharge was 15.69 ± 3.70 Hz.

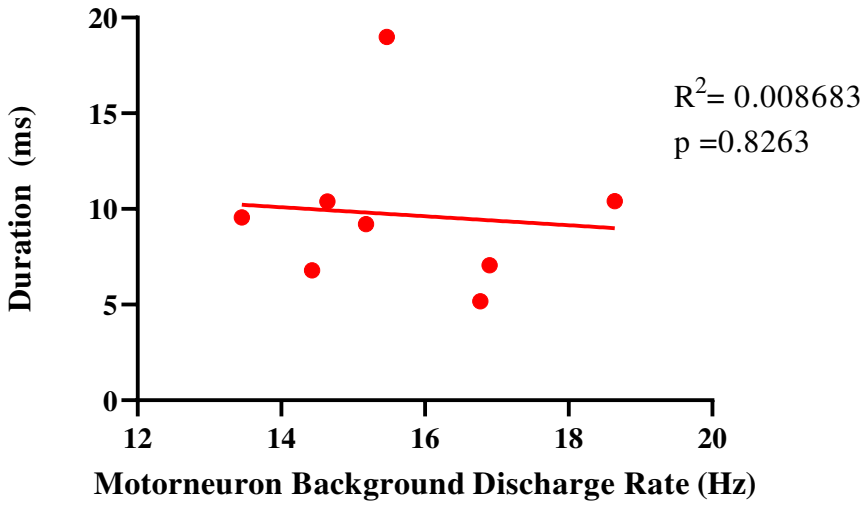

Figure 3.14. Masseter stretch reflex duration and background discharge rate relationship (n=8).

Transcranial Magnetic Stimulation (TMS) Experiments

27 SMUs from the tibialis anterior (TA) and 8 SMUs from abductor pollicis brevis (APB) were extracted (from 16 subjects and four subjects, respectively). Duration and latency of the motor evoked potential (MEP) were measured with PSF-CUSUM and PSTH-CUSUM, respectively (Figure 3.15)

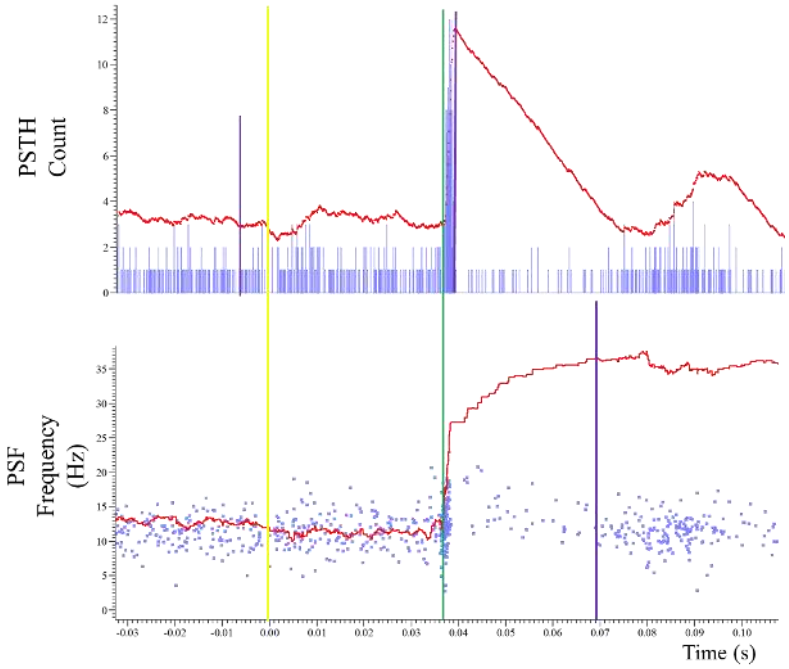

Figure 3.15. A sample data analyzed from a TMS experiment to estimate the EPSP profile.

PSTH and PSF analysis constructed from SMU analysis. The yellow line shows the electrical stimulus time point. Red lines are the CUSUM of each analysis, and the horizontal dashed lines are CUSUM-Error Boxes. Green lines demonstrate the latency of the excitation, and purple lines the end of the excitation. EPSP duration is the time interval between the purple and green lines. The SMU recordings were made from the TA muscle. The number of stimuli delivered in this experiment was 494, and the background firing frequency was 11.41 ± 2.44 Hz.

TA-MEP duration was measured 21.15 ± 15.28 ms with PSF-CUSUM, 7.54 ± 6.86 with PSTH-CUSUM, 9.44 ± 3.72 ms with MMU-CUSUM, and 15.55 ± 6.32 with SEMG-CUSUM (mean ± SD). PSTH-CUSUM was found significantly lower than PSF- and SEMG-CUSUM (Dunn, $p < 0.0001$). MMU-CUSUM was also found shorter than

PSF- and SEMG-CUSUM, significantly (p = 0.0342 and p = 0.0344, respectively).No significant difference was obtained in MEP latency between PSTH-CUSUM (30.53 ± 3.85 ms), MMU-CUSUM (30.87 ±3.54 ms), and SEMG-CUSUM (31.04 ± 4.60 ms) (Kruskal-Wallis, p= 0.1561, Fig 3.16a).

The mean MEP duration obtained from APB muscle was 15.40 ± 9.01 ms with PSF-CUSUM, 6.02 ± 1.55 ms with PSTH-CUSUM, 7.88 ± 2.12 ms with MMU-CUSUM, and 11.83 ± 2.77 ms with SEMG-CUSUM analysis. PSTH-CUSUM was found significantly shorter than PSF- and SEMG-CUSUM (p = 0.0277 and p = 0.0332, respectively). The latency of the MEP was also not significantly different between PSTH-CUSUM (22.79 ± 4.02 ms), MMU-CUSUM (22.81 ± 3.54 ms), and SEMG-CUSUM (21.44 ± 0.81 ms) (Kruskal-Wallis, p = 0.4084, Fig 3.16b).

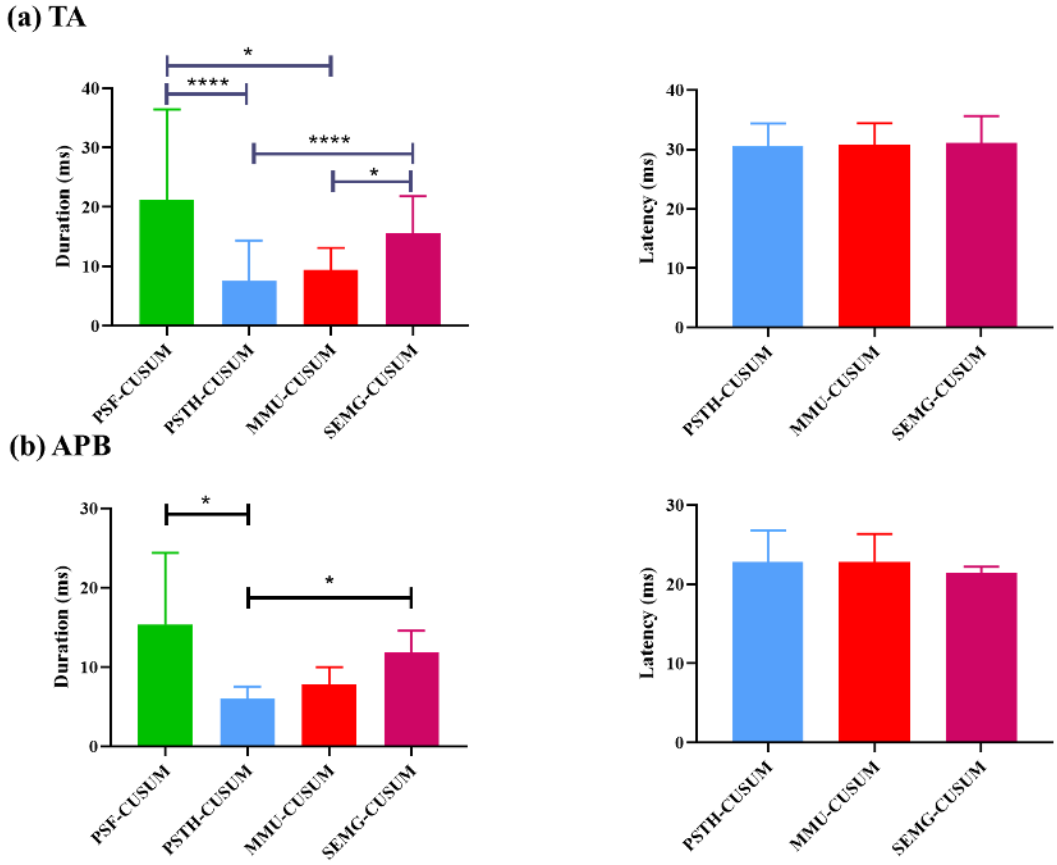

Figure 3.16. MEP duration and latency measurements with various analysis methods.

Recordings were made from (a) TA, (b) APB muscles. n=27 for TA and n=8 for ABP. ****p<0.0001,*p<0.05 and error bars are standard deviations.

MEP duration and background discharge rate were not significantly correlated with linear regression analysis from TA and APB muscle (p=0.0672 and p=0.5572, respectively) (Figure 3.16). TA average motoneuron background discharge was 9.27 ± 1.77 Hz, and APB 10.49 ± 2.90 Hz.

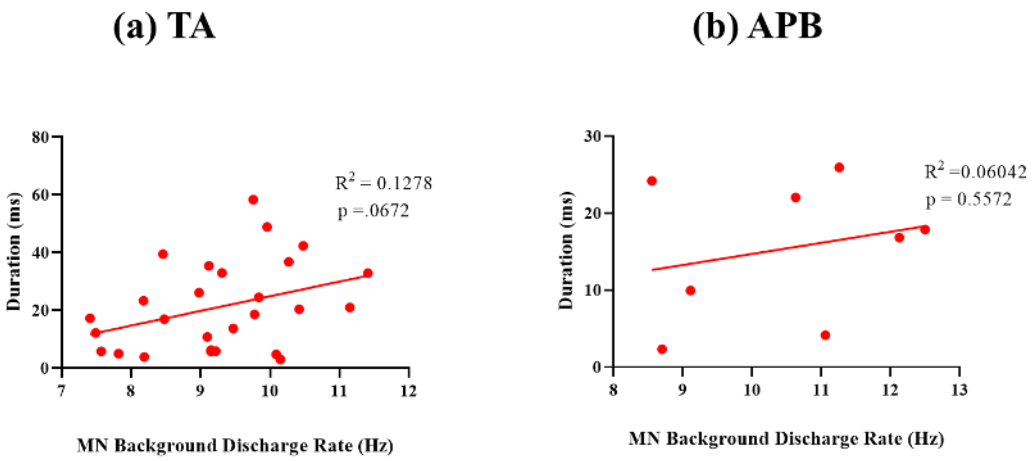

Figure 3.17. MEP duration and background discharge rate relationship.
Analysis were made from (a) TA (b) APB muscle recordings.

H-Reflex Experiments

Nine SMUs were analyzed from 6 subjects with PSF-CUSUM and PSTH-CUSUM to obtain the duration and latency of the H-reflex, respectively (Fig 3.18).

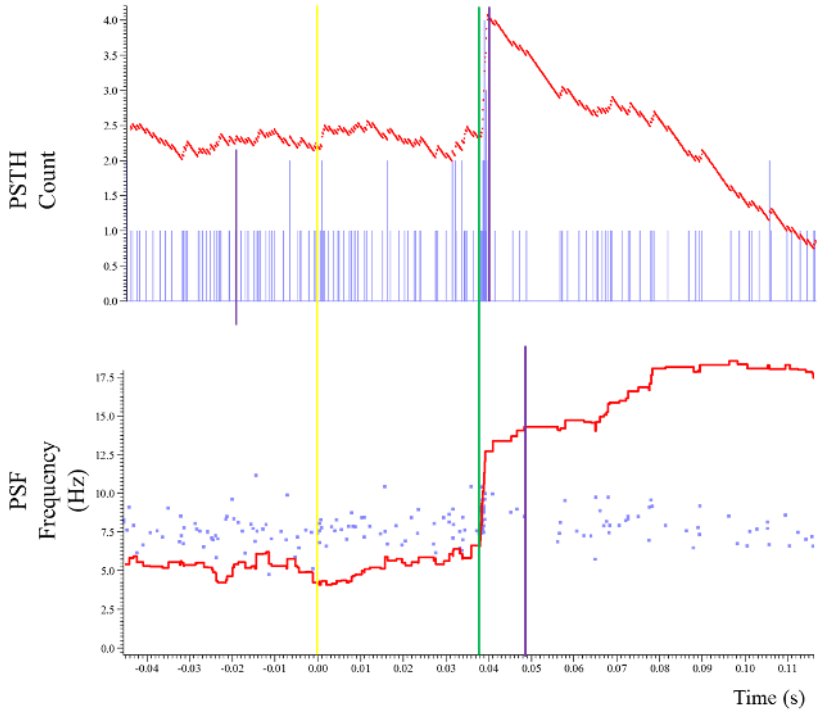

Figure 3.18. A sample H-reflex experiment analysis to estimate the EPSP profile. PSTH and PSF analysis built from SMU analysis. The yellow line shows the electrical stimulus time point. Red lines are the CUSUM of each analysis, and the horizontal dashed lines are CUSUM-Error Boxes. Green lines demonstrate the latency of the excitation, and purple lines the end of the excitation. EPSP duration is the time interval between the purple and green lines. The background firing frequency of soleus SMU analyzed in this experiment was 6.25 ± 1.17 Hz, and the stimulus number delivered from the tibial nerve was 189.

The PSTH-CUSUM duration (1.94 ± 0.53 ms) was obtained significantly shorther than PSF-CUSUM (13.03 ± 6.38 ms), MMU-CUSUM (10.07 ± 1.82 ms), and SEMG-CUSUM (11.20 ± 7.36 ms) (Dunn, $p = 0.0004$, $p = 0.0017$ and $p = 0.0106$, respectively).

H-reflex latency was detected 35.04 ± 1.83 ms with PSTH-CUSUM, 33.86 ± 2.41 ms with MMU-CUSUM, and 33.85 ± 2.05 with SEMG-CUSUM (mean ± SD). PSTH-CUSUM latency was found significantly higher than MMU- and SEMG-CUSUM (Dunn, $p = 0.0071$ and $p = 0.0005$, respectively).

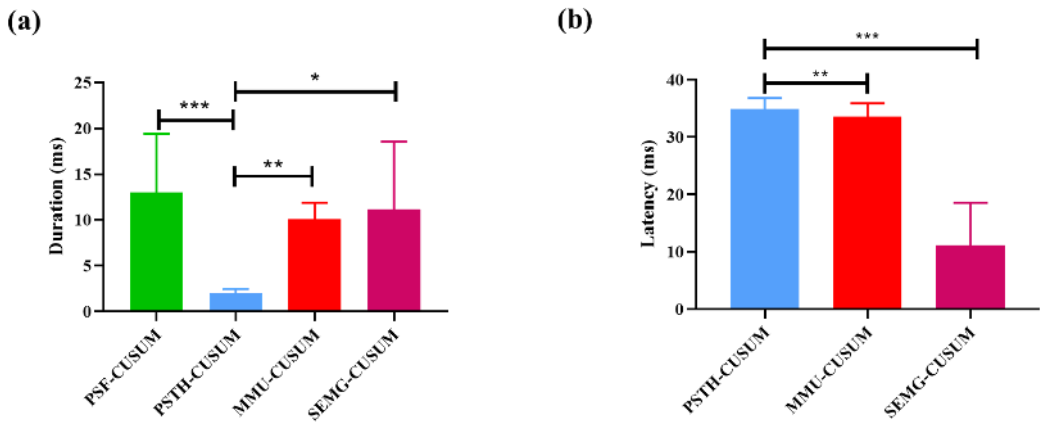

Figure 3.19. H-reflex duration and latency calculated with various analysis methods.
n=9, **p<0.05 and ***p<0.001 and error bars are standard deviations.

H-reflex duration and background discharge rate were not significantly correlated with linear regression analysis (p = 0.53) (Figure 3.16). The mean motoneuron background discharge was 6.25 ± 1.17 Hz.

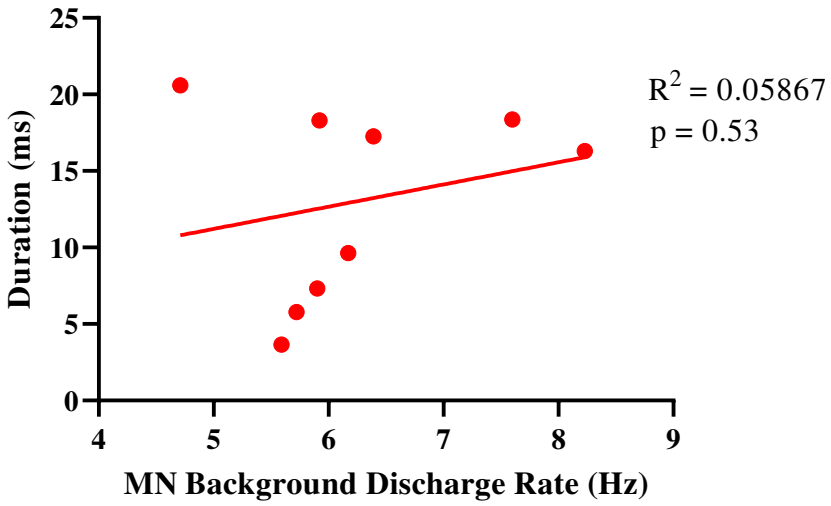

Figure 3.20. H-reflex duration and background discharge rate relationship (n=9).

CHAPTER 4
DISCUSSION

Here in this thesis, the duration change and the background discharge rate data were taken from the rat brain slice experiments to investigate their correlation. A significant correlation was found between the background discharge rate and duration with linear regression analysis in IPSP/EPSP injection experiments (Section 3.1). The duration of the inhibition increases as the background discharge rates decreases. This has been shown previously in human studies that the motoneurons with lower firing discharge rates were known to have higher inhibitability (Kudina, 1999). The duration of the excitation increases as the background discharge rate incremented as more of the falling phase of the EPSP can reach the threshold as shown in Figure 1.7. A model was proposed (Schwindt & Calvin, 1972) to explain the PSP changes with background discharge rate (sawtooth-like membrane potential model) shown in the Fig 4.1. According to this model, in a regularly firing neuron, sawtooth-like membrane potential reaches the FT, it fires, and then it hyperpolarizes (AHP) to approximately a ten mV potential from the FT. In our experiments, we use these frequency changes concerning the duration of the PSPs for the estimation using this model.

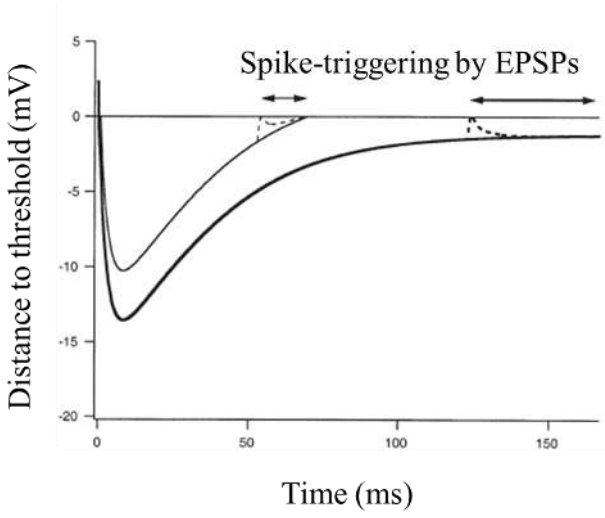

Figure 4.1. Different effects of the same EPSP on the motoneurons with different firing frequencies according to the sawtooth model.

Modified from Turker and Powers (2002).

We proposed the extrapolation approach earlier (Ozyurt et al., 2019) based on linear extrapolation of the background discharge rates to estimate the PSP duration at rest (zero Hz discharge rate). However, the primary purpose of using animal data and to construct the correlation plot between the discharge rate and duration was to obtain the motoneuron background discharge rate that delivers the exact PSP duration, which was predetermined to be 50 ms in the slice experiments. Fifty ms PSP duration was obtained at 2.88 Hz for IPSP, and 23.50 Hz for EPSP in the brain slice experiments. These data were used to estimate the duration of IPSP and EPSP on human motoneurons.

A discharge rate versus PSP duration correlation plot similar to rat slice experiments was constructed from three EPSP and three IPSP experiments performed on human subjects. PSF-CUSUM analysis was used to obtain the duration of PSPs since this method was shown to demonstrate the underlying PSPs more accurately than any conventional method used in the literature (Turker & Powers, 1999, 2003, 2005). Also, the duration data acquired from rat slice experiments were measured from PSF-CUSUM. The IPSP experiments on humans revealed a significant reverse relationship between the background discharge rate and duration of IPSP; however, in EPSP experiments, no significance was found.

The latency of the PSPs was detected with PSTH-CUSUM because previous studies revealed its success at indicating the initiation of the reflexes (Turker & Powers, 2003). In this thesis, the end of the PSP duration obtained from PSF-CUSUM. The duration of the PSPs was therefore obtained in combining PSTH and PSF methods.

Also, traditional methods (SEMG and MMU with CUSUM) were used to compare the duration and latency of the PSPs to compare with PSF-CUSUM and PSTH-CUSUM, respectively.

4.1. Evaluation of IPSP experiments

The opposite correlation was significant in all three experiments with the background discharge rate and firing frequency. The extrapolation approach was used to determine the duration of silent motoneurons. The 2.88 Hz value extracted from rat brain slice experiments was used to combine animal and human studies to acquire the estimated duration of IPSP in all three experiments.

4.1.1. Cutaneous Silent Period Experiments

Moderately painful electrical stimuli were delivered at the little finger, and reflex responses of FDI muscle (both stimulation and recording areas are innervated from the

C8 dermatome) single motor units and SEMG were recorded. Ten times sensory threshold was chosen (Kofler, Leis, & Valls-Sole, 2019; Shefner & Logigian, 1998), and this intensity created CSP in all experiments we have performed. PSTH- and PSF-CUSUM charts were constructed to investigate the latency and the duration of the CSP inhibition. The underlying IPSP duration was decided to evaluate with PSF-CUSUM since the PSTH-CUSUM has been shown substantial errors in detecting the duration of the IPSPs (Turker & Powers, 1999, 2003). Therefore, the duration found by PSTH-CUSUM and MMU-CUSUM was only used for comparison with the literature. SEMG recordings were analyzed by spike-triggered average method, and CUSUM analysis was also performed; however, SEMG-CUSUM failed to show the inhibition since the stimulus artifact was too large. Therefore, it prevented obtaining the CSP latency and duration. This was unavoidable as the stimulation points, and SEMG electrodes were nearby.

The original study that used PSF-CUSUM analysis to discover CSP characteristics has shown that the duration of the inhibition was measured to be significantly higher with PSF-CUSUM compared to PSTH-CUSUM and SEMG-CUSUM (Kahya, Yavuz, & Turker, 2010). Consistent with this study, the duration of the PSTH-CUSUM was found significantly lower than PSF-CUSUM in this thesis ($p<0.0001$). The excitation seen after CSP in PSTH-CUSUM was a part of the rising phase of the inhibition revealed with PSF-CUSUM analysis (Figure 3.3). The spikes delayed due to inhibitory stimulus occur in the rising phase of the inhibition, interpreted as excitation in PSTH-CUSUM.

Due to these reasons, the duration obtained from PSF-CUSUM was used in linear regression analysis to investigate the relationship between background firing rate and duration. A significant reverse correlation was found between the background firing rate and duration ($p=0.0016$). That result matched our hypothesis and previous studies (Kudina, 1999; Turker & Cheng, 1994).

The extrapolation approach we proposed before (Ozyurt et al., 2019) was used here to detect the duration of the MNs in the rest. Since these recordings were indirect and they were affected by background discharge rate, it is thought that the silent MNs were less contaminated and can reveal a better estimation of CSP. The IPSP duration of these silent (“0 Hz”) MNs was found 219.90 ms.

The motoneuron background discharge rate which delivered the exact PSP duration in the brain slice experiments, was 2.88 Hz. This value was now put into the linear regression equation, and 192.91 ms of estimated IPSP duration was found.

CSP is beneficial in clinical use to investigate the components of the sensory nerves when other traditional electrodiagnostic methods fail. The hallmark of this method is; it is convenient to evaluate the thinly myelinated, small diameter A-delta afferents and their spinal connections with motor pathways (Kofler, Leis, & Valls-Solé, 2019). The latency and duration of the CSP were used for the assessment of conduction abnormalities (Leis, 1994), progression of the motoneuron diseases (Castro, Swash, & de Carvalho, 2021)), mechanism of neurodegenerative diseases (Cengiz, Mercan, & Kuruoglu, 2018), and evaluation of the effects of the drugs in Parkinson's Disease (Serrao et al., 2002; Urasaki, Miyagi, & Kishimoto, 2021).

Due to the abovementioned wide area of utilization, CSP is an essential tool for clinics and research. Our methods in this study can help estimate CSP more reliably.

Renshaw Inhibition (RI) Experiments

The RI experiments presented in this thesis are a part of our published paper (Ozyurt et al., 2019).

The thickest motor axons were electrically stimulated, and low-threshold, voluntarily active SMUs were recorded to reveal the synaptic profile of the Renshaw inhibition. The stimulation-induced a direct motor response (M-response) on the entire muscle and indirect inhibition on the voluntary activated SMUs.

Previously, the studies on human subjects to find the Renshaw inhibition characteristics were based on paired H-reflex stimulations (Pierrot-Deseilligny & Bussel, 1975). However, this method probably also causes an Ib inhibition, whose latency is similar to the RI. Thus, this inhibition obtained using the pair H-reflex method prevents the correct measurements of RI (Delwaide & Oliver, 1988). Also, the stimulus used in those experiments was so frequent that it could cause presynaptic inhibition in the primary afferent endings. Due to these reasons, the RI can be misevaluated using the traditional methods. The significance of the M-only stimulation method used in this thesis is that it contains minimum contamination from other reflex pathways (Ozyurt et al., 2019); therefore, it exhibits a more reliable RI profile.

The RI duration obtained with PSF-CUSUM was significantly longer than PSTH- and MMU-CUSUM ($p<0.0001$). PSF-CUSUM duration was also longer than the studies that used the classical paired H-reflex method (Hultborn & Pierrot-Deseilligny, 1979; Rossi, Mazzocchio, & Decchi, 2003).

Linear regression of the RI duration and MN background firing rate revealed a significant inverse relationship ($p=0.0076$). Shorter RI durations were found when MNs

were firing at higher background discharge rates. This may be since the high excitability makes them less responsive to IPSPs. The resting motoneuron duration was found at 53.55 ms by the extrapolation approach. These RI experiments data in this thesis were published, and it was the first time we described the extrapolation approach (Ozyurt et al., 2019). Going one step further, the animal and human studies were put together in this thesis to make a better estimate of RI duration. The correct discharge rate to determine the IPSP duration, as the animal work tells us, is 2.88 Hz, and by using this value, the estimated duration was found 43.94 ms in human experiments. Thus, we believe that we are now closer to the actual RI duration in human neuronal circuitries. The optimized techniques used here can be applied to clinics for evaluating the effect of RI in pathological conditions or diseases.

4.1.2. The Masseter Inhibition Experiments

Eight times sensory threshold in 0.05 ms pulse width were delivered from the lip area to elicit a potent masseter inhibition on voluntarily activated SMUs. The stimulus intensity was generated apparent inhibitions on all 14 SMUs we analyzed.

The high-intensity stimulus created a short latency and a long-latency inhibition in the masseter muscle with PSTH-CUSUM analysis (Miles et al., 1987). However, Uginčius et al. (2017) has found that with PSF-CUSUM analysis, lip stimulation produced a single long-lasting or a compound inhibition which was continued with a long-lasting excitation. Although we have discovered compound inhibitions in 6 SMUs consistent with the Uginčius et al. (2017) study, we decided to evaluate this compound inhibition as one long-latency since the end of the first inhibition and the beginning of the second inhibition was not too apparent in all the 6 SMUs with PSF-CUSUM analysis. Different stimulus intensities must be delivered to elicit these two inhibitions with an increased number of subjects. In resting 8 SMUs, we have found a long-lasting inhibition. These experiments will be continued in the future to analyze the two phases in the masseter inhibition separately in detail.

The duration of the inhibition was detected longer with PSF-CUSUM compared to the probability-based methods (PSTH- and MMU-CUSUM) as anticipated.

MN background firing rate significantly affected masseter inhibition with linear regression analysis ($p = 0.0212$). Their relationship was similar with animal brain slice studies and other IPSP experiments. That is, the duration shortens with increased SMU firing frequency. Thus, a 191.20 ms inhibition duration was observed in silent MNs by

extrapolation approach, and 172.04 ms of duration was calculated using the animal slice experiment firing rate data at 2.88 Hz.

The main aim was to induce A-delta afferents by moderately painful lip stimulation in this study, but this stimulation also activates the lower threshold Aβ mechanoreceptor fibers. It was hypothesized that the first phase of the inhibition might arise from these Aβ mechanoreceptor pathways and the second by the nociceptive pathways (Uginčius et al., 2017). For better estimation of these inhibitory pathways, the duration of the two phases will be investigated in further experiments using this thesis's approaches.

As the PSF-CUSUM method was shown to upgrade the conventional techniques, the extrapolation and direct-indirect method combination for masseter duration estimation will further improve. Thus, it can be an effective tool for reassessing the trigeminal nerve pathways in this region's normal physiological conditions and diseases.

4.2. Evaluation of EPSP experiments

Although studies on rat human hypoglossal neurons revealed a significant relationship between the background discharge rate of the SMUs and the duration of the excitation, no significance was observed in any human studies performed in this thesis. In direct studies on rat slices, the results were not affected by other mechanisms since the MNs were out of their physiological environment. Human recordings are vulnerable to interference from different pathways; the excitatory stimulus can also induce the inhibitory mechanisms nearby, and the overlaps of their activation can restrain the excitatory responses.

In addition to that, slice studies are well controlled compared to human studies; for instance, it is possible to fire motoneurons in a wide range of frequencies by injecting current transient in different amplitudes and time courses; however, in human MNs, the firing frequency of the MNs is restricted. This is especially important in excitatory experiments since obtaining the falling phase of the EPSP is difficult in human studies and finding the optimal stimulus for that is not always easy. Therefore, detecting the falling phase of the EPSP could be possible if an SMU can discharge at a broad range. Unfortunately, this is hard for the subjects to bring out and sustain; this is not possible for all muscles (some muscles have a narrower range of firing frequencies).

Acquiring the falling phase of an EPSP is troubled because the rising phase of the EPSP crosses the firing threshold easily since it rises very fast and is larger than the synaptic noise. The falling phase of the EPSP can only cross the threshold if this rapidly rising first phase fails to pass the threshold, and additionally, synaptic noise helps it to

cross. This event is infrequent; thus, this constitutes one of the significant issues gaining the complete EPSP profile in this thesis. We only obtain the falling phase of the excitation only in a limited number of SMUs.

4.2.1. Masseter Stretch Experiments

A brief tap induced the masseter stretch reflex to the chin by a reflex hammer. Although we have performed several experiments in only four experiments from two subjects, we elicit the stretch responses distinctly.

Masseter stretch reflex duration was calculated 9.70 ± 4.20 ms with PSF-CUSUM, and the latency was 11.25 ± 0.96 ms with PSTH-CUSUM in 8 SMUs. Although the reflex latency was consistent with the previous studies (Murray & Klineberg, 1984), there are no data to compare the duration since no other studies used PSF-CUSUM to obtain this reflex. The background discharge rate and the duration of the reflex exhibit no correlation in linear regression analysis ($p = 0.8263$).

The subjects were asked to isolate an SMU during the experiments; when this SMU fire sustainably, the tap stimulus intensity was optimized. The optimal stimulus was decided until this SMU occur in an average of 3 out of 10 stimuli at the same latency with masseter stretch reflex. The occurrence of the SMUs at the expected time interval was monitored from a monitor throughout the experiments to arrange the stimulus intensity. The effective stimulus was also calculated from the PSTH analysis from all experiments and found approximately 27%. This intensity was chosen to be optimal to exhibit the complete profile of the synaptic profile of masseter stretch. If the intensity was too high, it is not possible to see the falling phase of the EPSP. Also, high stimulus causes the "field potential," which means the aggregation of SMUs; thus, it is hard to obtain the SMU we track isolated, and this causes miscalculations. On the other hand, if the intensity were too low, the EPSP would be unclear with the synaptic noise interference. In addition, we applied 347.25 ± 139.80 stimuli (mean ± SD) to increase the possibility of catching the complete profile of EPSP. Despite these optimizations, we still failed to observe the falling phase of the EPSP in 6 out of 8 SMU recordings, and for those two we observe that the falling phase was not sufficient.

The periodontal mechanoreceptor system was known to induce synaptic potentials on masseteric motoneurons (Türker, 2002). The quick taps to the tooth were shown to induce an inhibitory response on the masseter muscle, which comes from the periodontal receptors; it is thought to be a protective mechanism for teeth and jaws

(Türker, Brodin, & Miles, 1994). Therefore, along with the masseter stretch reflex, the stimulus applied in these experiments can also activate the periodontal mechanoreceptors; thus, an inhibition occurs with the stretch reflex. As shown in Fig 3.12 in the Results section; a potent inhibition was seen after the stretch response. Perhaps this interference prevents the occurrence of the falling phase of the reflex.

Furthermore, the dental cotton wools were placed between the molar teeth to avoid hitting the jaws. This was thought to avoid the activation of periodontal receptors; however, the inhibition still occurs. Perhaps the best solution to this problem would be the fixation of the jaw with a special set up and/or blocking the mechanoreceptors with local anesthetics.

Transcranial Magnetic Stimulation (TMS) Experiments

The MEP response of TMS was recorded from TA and APB muscle to reveal the EPSP profile. Stimulus intensities evoke between 10 and 50 SMUs out of 100 (the time interval where MEPs anticipated) were accepted. This stimulus intensity optimization was performed to estimate the EPSP profile thoroughly. This part of the thesis includes data from our published study, which presents the cortical silent period characteristics analyzed by PSF-CUSUM (Özyurt et al., 2019). The cortical silent period (CSP) is decreased EMG activity after motor evoked potential (MEP). This period is reevaluated with PSF-CUSUM and found that the initial inhibitory segment of CSP represented with traditional methods can be the falling phase of the EPSP since the activity here was found higher than the average of the background discharge rate. Therefore, this thesis suggests a better presentation of the EPSP profile compared to traditional methods. Also, in this thesis, we have compared the conventional techniques with PSF-CUSUM and observed significant differences with PSTH-CUSUM in the duration of the MEP in both TA and APB recordings.

Although the TMS was presented a preferable EPSP profile (in both APB and TA muscle recordings) than other EPSP experiments we performed, the correlation between the background discharge rate and duration of MEP was not significant with linear analysis. The results we obtained were probably not sufficient to detect the entire profile of the EPSP; therefore, the linear regression analysis fails to represent the relationship between the background discharge relationship and duration. On the other hand, we have observed a significant association of duration and background firing frequency in our previous paper (Özyurt et al., 2019).

In addition to that, we compared the effective stimulus intensity with duration (data not shown), and no significant difference was found between them. So perhaps the stimulus intensity was unsuitable, or maybe it could be arranged in a broader range in each experiment to procure the falling phase.

4.2.2. H-reflex Experiments

The H-reflex was elicited on the soleus muscle through tibial nerve stimulation. The optimal stimulus intensity was determined as three out of ten consecutive stimuli that the SMUs occur where H-reflex was expected to appear, 30-45 msec (Miles et al., 1989). This was determined the optimal stimulus intensity revealing the EPSP characteristics.

Nine SMUs were analyzed, and PSTH-CUSUM duration was significantly lower than PSF-CUSUM and other traditional analysis methods we tested.

The linear regression analysis demonstrates no correlation between the duration and background motor unit discharge rate. The optimal stimulus intensity was below our expectation (16.11% ± 4.9); nevertheless, we observed the H-reflex in all 9 SMUs analyzed. Unfortunately, any of them presented the falling phase of the EPSP. Thus, further experiments will be done with more controlled stimulus intensities.

CHAPTER 5
CONCLUSION

Postsynaptic potentials (PSPs) have been using for years to investigate the physical structure and function of the neuronal circuitries. Electromyography (EMG) is an effective tool to study PSPs on human subjects; however, all recordings performed on humans have to be indirect. Therefore, the direct recordings collected from animal slice experiments were used in this thesis to evaluate PSP characteristics more precisely.

Additionally, the experiments performed on human subjects were analyzed by probability-based methods that have been shown erroneous to detect the genuine profile of the PSPs (Turker & Powers, 2005). In this thesis, the discharge-rate-based-method PSF-CUSUM was used to evaluate the PSP characteristics, which are more accurate than the probability-based methods in animal slice studies (Turker & Powers, 2003). However, the probability-based methods (PSTH-, MMU- and SEMG-CUSUM) were used in this thesis for comparison with the literature findings. Previous studies have shown significantly different PSP values from PSF-CUSUM findings in most of the experiments.

The background discharge rate effect on the duration of PSPs was investigated in previous studies (Ashby & Zilm, 1982a). The SMUs that fire at a lower rate has been found to have higher inhibitability (Kudina, 1999). In this thesis, the background discharge rate effect on the PSP duration was also analyzed using the rat brain slice data. Based on the brain slice findings, human experiments and further analysis were performed to obtain the actual duration of the PSPs. These additional analyses include using the extrapolation method and integrating our results with the findings on brain slice findings to estimate PSP duration from linear regression analysis.

Using the extrapolation approach on human experiments was one of the major novelties of this thesis to estimate the duration of the PSPs in resting motoneurons. This method was firstly described in our previous research, which was also a part of this thesis (Ozyurt et al., 2019). Using this approach, the indirect human recordings would be free of the background discharge rate effect by extrapolating the silent/resting motoneuron duration; thus, a purer estimation of the PSP profile could be obtained.

The main aim was to combine direct animal brain slice studies with indirect human studies. In order to do that, three EPSP and three IPSP experiments were performed on humans, and the analysis corresponding to rat brain slice analysis was done for this integration. Although the rat slice studies revealed a significant relationship between motoneuron background discharge rate and duration of the EPSP and IPSP, we only found this significance in the IPSP experiments (Masseter inhibition, Renshaw inhibition, and CSP experiments). Extrapolation and direct-indirect recording combinations were performed on these experiments, and IPSP durations were estimated.

The background effect on EPSP duration was not significant, probably because obtaining the falling phase of the EPSP was a great challenge. Most of the SMUs in this thesis could not discharge at high enough rates to obtain the details of the EPSP falling phase. Thus, more experiments are necessary with rearranged stimulus intensities.

All human experiments performed in this thesis are widely used in research, diagnostics, investigation, and disease progression; therefore, the novel approach for a better estimation of PSP duration in this thesis would be beneficial to the literature. Further developments and experiments must be needed for especially for EPSP experiments. Following the standardization in healthy individuals, these methods can also be used on patients with neuromuscular diseases or spinal cord injuries to rewire the nervous system in diseases.

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APPENDIX A

Sample Informed Consent Form (Turkish)

The participants were informed about the experiments and their requirements verbally; in case of approval, they were asked to read the informed consent form, fill the questions related to their health condition, and signed the consent form below.

The consent form below was arranged accommodated to thesis format and it may include format differences.

Bilgilendirilmiş Gönüllü Olur Formu

Araştırmanın Adı: İnsan beyninin ve iskelet kasının elektriksel aktivelerinin araştırılması
Protokol no: 2013.066.IRB2.33/11.02.2013

LÜTFEN DİKKATLİCE OKUYUNUZ !!!
Bu çalışmada yer almayı kabul etmeden önce çalışmanın ne amaçla yapılmak istendiğini anlamanız ve kararınızı bu bilgilendirme sonrası özgürce vermeniz gerekmektedir. Size özel hazırlanmış bu bilgilendirmeyi lütfen dikkatlice okuyunuz, sorularınıza açık yanıtlar isteyiniz.

ÇALIŞMANIN AMACI NEDİR?

Bu çalışmada insan iskelet kaslarının santral ve periferal kontrolünün incelenmesi amaçlanmaktadır. Bu çalışmada, beynin elektriksel aktivitesi ve kaslarda ve farklı uyarılarla oluşturulan refleks yanıtları yeni bir analiz yöntemi ile tekrar incelenecektir. Klasik olarak literatürde olasılık bazlı yöntemden kaynaklanan sinir kas sistemi sinir ağlarındaki yanlışlar bizim ileri sürüp doğruluğunu ispatladığımız frekans analizi metodu ile tekrardan değerlendirilecek ve doğru sinir haritaları ortaya konulacaktır. Ayrıca EEG ve EMG nin ne derece birbirlerini etkilediklerinin anlaşılması konularında çalışmaları da amaçlarımız arasındadır.

Bu deneyler, insandaki sinir yolaklarının doğru haritalarını ortaya çıkaracağı için, sinir/kas hastalıkların klinik tanı ve sağaltımlarının geliştirilmesi için bilimsel bir temel oluşturacaktır.

KATILMA KOŞULLARI NEDİR?

Bu çalışmaya dâhil edilebilmeniz için anestetik maddelere hassasiyeti olmayan ve herhangi bir nörolojik veya kas bozukluğu olmayan sağlıklı erişkin birey olmanız gerekir.

NASIL BİR UYGULAMA YAPILACAKTIR?

- Çalışma Koç Üniversitesi Tıp Fakültesi Nörofizyoloji laboratuarlarında, Koç Üniversitesi Hastanesi Nöroloji veya Marmara Üniversitesi Pendik Eğitim ve Araştırma Hastanesi Nöroloji laboratuvarlarında yapılacaktır.
- Kas kasılması beyin tarafından kontrol edilir. Bir kasınızı kasmaya karar verdiğinizde beyin sinir hücrelerinden elektriksel sinyal omurilik boyunca motor sinirlerle kasa kadar gönderilerek kasılma sağlanır. Kasılma hareketine reflekslerle ayar ve düzenleme yapılır.
- Katılacağınız deneyde aşağıdaki prosedürler tüm deneyler için aynıdır:

  - Deri yüzeyinden yapılacak kayıt için cilt %70 lik alkol ile temizlenecek, deri yüzeyi aşındırıcı jel ile hafifçe ovulacak ve yüzeyel elektrotlar kasın liflerine paralel gelecek şekilde deri üzerine yapıştırılacaktır. Başdaki saçlı deri üzerine elektrotlar yapıştırılırken sadece jel kullanılacaktır.
  - Kas içinden yapılacak kayıtlarda, cilt %70 lik alkol ile silinecek ve elektrot telleri kas içine 25G kalınlığında bir enjektör iğnesi yardımı ile yerleştirilecek, bu iğne daha sonra geri çekilip, teller kasın içinde bırakılacaktır ve kasınızı birkaç defa kasmanız istenecektir. Yerleştirilen teller deney sırasında ağrıya neden olmazlar ve deney sonunda da kolayca ağrısız olarak çekilip çıkarılacaktır. Elektrotlar steril ve tek kullanımlıktır.
  - Kayıt esnasında sizden karşınızdaki ekrana bakarak istenen derecede kaslarınızı kasmanız istenecektir.
  - Çalışmamız baş bölgesinde çiğneme ve mimik kaslarını; kol bölgesinde üst kol, alt kol ve el kaslarını ve bacak bölgesinde ise baldır ve bacak kaslarını içerecektir.
  - Her bir deneyde sadece bir bölgeniz uyarılacak ve o bölgedeki bir ya da iki kastan kayıt alınacaktır. Uyarılar elektrik ve mekanik olduklarında 1-3 saniye aralıklı olarak yaklaşık 300 adet, lazer olduklarında 10-15 saniye aralıklı 50 kere, transkranyel beyin uyarılarında ise 4-6 saniye aralıklı olarak yaklaşık 300 adet olacaktır.
  - Deneyler hazırlık aşaması ile birlikte en fazla üç saat sürecektir.
  - Bir kişide aşağıda detayları verilen deneylerden sadece bir tanesi yapılacaktır.

KATILACAĞINIZ DENEYDE AŞAĞIDAKİ PROSEDÜRLERDEN SADECE İŞARETLENMİŞ OLAN DENEY UYGULANACAKTIR. UYGULANACAK PROSEDÜRLER HAKKINDA GEREKLİ BİLGİLER AŞAĞIDA VERİLMİŞTİR.

☐ Transkranial Manyetik Uyarı

- Transkranial manyetik stimülasyon (TMS) beynin ağrısız ve invazif olmayan bir şekilde uyarılarak beyinden kaslara giden yolakların araştırılması için kullanılan bir yöntemdir.
- Katılacağınız bu deneyde motor kortekse verilen manyetik uyarının kas hareketi ve hareketsiz durumlardaki etkileri incelenecektir.
- Uyarı esnasında belli kaslarınızda kasılma veya seğirme hissedebilirsiniz. Uyarılar arası aralık en yakın dört saniye ve tek tek verilen (single pulse) manyetik uyarı ağrısız ve zararsızdır.
- Magnetik koiller klasik sekiz seklinde 15 x 8 x 1 cm ebatlarinda; yuvarlak koil ise 13 cm capli ve 2 cm kalinliktadir.

☐ Mekanik Uyarı

- Katılacağınız bu deneyde kaslara, tendonlara ya da dişlere verilen uyarı ile oluşan refleks yanıtlar ve elektriksel özellikleri incelenecektir.
- Mekanik uyarı esnasında belli kaslarınızda kasılma veya seğirme hissedebilirsiniz. Mekanik uyarı ağrısız ve zararsızdır.
- Eğer mekanik uyarı dişinize verildi ise, deney sırasında uyarı verilen dişinizin çevresine lokal anestetik madde (Diş Hekimlerinin kullandığı anestetik madde ve uygulama yöntemi ile) verilerek geçici bir süre için (2 saat kadar) hissiz hale getirilecektir. Bu aynen diş hekimine gittiğinizde yapılan enjeksiyondur ve hiçbir yan etkisi olmayıp, etkisini iki saat içerisinde tamamen kaybeder. Dişiniz hissiz halde iken deney tekrarlanarak aradaki refleks farkı elde edilecektir.

☐ Lazer Uyarısı

- Katılacağınız bu deneyde lazer uyarı ile oluşan refleks yanıtlar ve elektriksel özellikleri incelenecektir.
- Lazer uygulaması küçük bir alana (yaklaşık 5mm^2) yapılacaktır. Kalıcı bir etkisi olmayıp, hafif rahatsızlık ve sıcaklık hissi verebilir.

☐ Elektriksel Uyarı

- Katılacağınız bu deneyde elektrik uyarı ile oluşan refleks yanıtlar ve elektriksel özellikleri incelenecektir.
- Uyarı esnasında belli kaslarınızda kasılma veya seğirme hissedebilirsiniz. 2-100 mA seviyesinde verilen elektriksel uyarılar deride hafif kaşınma hissi ve rahatsızlık oluşturabilir. Bu uyarıların hiçbir kalıcı yan etkileri olmayıp tamamen zararsızdırlar.

SORUMLULUKLARIM NEDIR?

Araştırma ile ilgili olarak nörolojik, dental ya da bir kas hastalığınız olup olmadığı ve anestetik maddelere hassasiyetiniz olup olmadığı konularında sorumlu araştırıcıyı bilgilendirmek sizin sorumluluğunuzdur. Bu koşullara uymadığınız durumlarda araştırıcı sizi uygulama dışı bırakabilme yetkisine sahiptir.

KATILIMCI SAYISI NEDİR?

Araştırmada yer alacak gönüllülerin sayısı 100 kişidir.

KATILIMIM NE KADAR SÜRECEKTİR?

Bu araştırmada yer almanız için öngörülen süre yaklaşık 3 saattir.

ÇALIŞMAYA KATILMA İLE BEKLENEN OLASI YARAR NEDİR?

Bu çalışma araştırma amaçlı olduğu için size herhangi bir yararı olmayacak ve ancak insandaki sinir yolaklarının bulunmasını sağlayacağı için, ileride, kassal ve sinirsel hastalıkların klinik tanı ve sağaltımlarının geliştirilmesi için bilimsel temel oluşturacaktır. Bu yöntem insanda uyarı ile kaslarda ortaya çıkan potansiyeller incelenerek sinir haritalarının (sinir elektrik devrelerinin) ortaya çıkartılması mümkün olabilecek ve yeni yöntem ile bu devreler hatasız olarak yeniden çizilecek ve klinik nörolojide güvenle kullanılması sağlanacaktır.

ÇALIŞMAYA KATILMA İLE BEKLENEN OLASI RİSKLER NEDİR?

- Dişe ya da kaslara uygulanan mekanik ve elektrik uyarının, transkranial manyetik uyarının, lazer uyarının ve deri yüzeyinden yapılan kaydın sağlık açısından hiç bir sakıncası bulunmamaktadır. Kas içinden yapılacak kayıtlar ise, herhangi bir hastalık için yaptırdığınız iğneden (enjeksiyon) farklı değildir ve sağlık açısından hiç bir riski yoktur.

- Ender durumlarda iğne deliğinin yerinde enfeksiyon ya da küçük bir kan pıhtısı olabilir. Olası bir soruna karşı gerekli tedbirler tarafımızdan alınacaktır.

- İğne batırma yerlerinde sterilite kurallarına uyduğumuz için 30 senelik uygulamalarda herhangi bir şekilde cilt enfeksiyonu görülmemiştir. Ancak bu durum bir olasılık olduğu için etik kurul müracaatlarında söylenmektedir. Eğer bir cilt enfeksiyonu ile karşılaşır isek bölgeyi antiseptik (batticon) ile temizleyip üzerine antibiyotik pomat (furacin) sürüp gelişmesini takip edeceğiz. Ayrıca Koç Üniversitesi kampus içerisinde bulunan sağlık merkezindeki doktor ile bu konuyu görüşerek tedavinin devamı için konsültasyon yapacağız.

- Lokal anestezi enjeksiyonu, diş hekiminin size yaptığı enjeksiyondan farksızdır ve etkisi iki saat içerisinde tamamen geçer. Lokal anestezi hissi ortadan kaldırdığı için dilinizi ısırıp yaralayabilirsiniz. Bu konuda, diş hekimi olan Prof. Türker size lokal anestezi vermeden önce size lokal anestezi geçmişiniz hakkında sorular soracak, dişlerinizi muayene edecek ve gerekli tüm önlemleri alacaktır. Ayrıca lokal anestezi enjeksiyon ile verildiği için yukarıda iğne batırma ile ilgili sterilite gibi önlemler de alınmaktadır.

- Lazer kullanılan deneylerde hem araştırmacı, hem de gönüllü lazer ışınlarından gözlerini korumak için özel gözlük kullanacaktır.

Yine de bu konuda danışmak istediğiniz bir konu veya karşılaştığınız bir sorun olur ise, (212) 338 1174 numaralı telefondan Prof. Dr. Kemal Türker ile iletişim kurabilirsiniz.

ÖNCELİKLE AŞAGIDAKİ SORULARI YANITLAYINIZ, EĞER HERHANGİ BİR SORUYA EVET CEVABI VERDİYSENİZ ARAŞTIRMACIYA BİLDİRİNİZ.

GÜVENLİK TARAMASI

Lütfen aşağıdaki sorulara cevap verin.
Herhangi bir nörolojik ya da kas hastalığınız var mı?

☐ Evet
☐ Hayır

Lokal ya da genel anestetik maddelere alerjiniz var mı?

☐ Evet
☐ Hayır

Eğer yukarıdaki sorulardan herhangi birine evet cevabı verdiyseniz lütfen detayları aşağıya belirtiniz.

_____
_____

Son 48 saat içerisinde herhangi bir ilaç kullandınız mı?

☐ Evet
☐ Hayır

Cevabınız evet ise kullandığınız ilaçları belirtiniz

_____
_____

Sürekli kullandığınız bir ilaç var mı?

☐ Evet
☐ Hayır

Cevabınız evet ise kullandığınız ilaçları belirtiniz

_____
_____

Daha önce sara nöbeti, inme, ciddi kafa travması ve buna bağlı ameliyat veya beyinle ilgili başka bir hastalık geçirdiniz mi?

☐ Evet
☐ Hayır

Cevabınız evet ise belirtiniz

_____
_____

Başınızda (ağız içi hariç) şarapnel parçası, cerrahi klip, metal malzeme, vücut içine konulan kardiyak pacemaker, koklear implant, tıbbi pompa veya kalp içi tel gibi cihazlar mevcut mu?

☐ Evet
☐ Hayır

Cevabınız evet ise belirtiniz

_____
_____

Sık veya ciddi baş ağrısı yakınmanız, gebelik veya gebe olma ihtimaliniz veya ailenizde epilepsi hastası var mı?

☐ Evet
☐ Hayır

Cevabınız evet ise belirtiniz

HANGİ KOŞULLARDA ARAŞTIRMA DIŞI BIRAKILABİLİRİM?

İlaç alerjinizin olması, nörolojik veya kas sistemi ile ilişkili herhangi bir hastalığınızın olması durumunda veya çalışma programını aksatmanız nedeniyle doktorunuz sizi çalışmadan çıkarabilir.

Biz deneklerimizin kaslarında güçsüzlük, duyu kaybı, parmak uçlarında karıncalanma gibi belirtilerin olup olmadığını ve ailede bu şekilde kişilerin olup olmadığını sormaktayız. Ayrıca ailede şeker hastalığı, yüksek tansiyon olup olmadığı da sorulacak ve kişinin el ve ayak kasları, yürüyüşü ve dengesine bakılarak kontrol edilecektir. Deneylerimiz herhangi bir şekilde sinirlere zarar verecek bir yöntem içermediği için detaylı anamnez dışında sadece şüpheli gördüğümüz durumlarda sinir iletimi hızı ölçümü yapacağız.

TRANSKRANİAL MANYETİK STİMULASYON (TMS) DENEYLERİNE KATILACAKLAR İÇİN:

TMS' ye bağlı herhangi bir yan etki yaşamışsanız, sara nöbeti, inme geçirmişseniz, ciddi kafa travması ve buna bağlı ameliyat veya beyinle ilgili başka bir hastalık geçirmişseniz, beyin hasarına neden olan bir hastalık geçirmişseniz, başında (ağız içi hariç) şarapnel parçası, cerrahi klip, metal malzeme mevcutsa, vücut içine konulan kardiyak pacemaker, koklear implant, tıbbi pompa veya kalp içi tel gibi cihazlar

mevcutsa, sık veya ciddi baş ağrısı yakınmanız varsa, gebelik veya gebe olma ihtimaliniz varsa, ailede epilepsi hastası varsa çalışmadan çıkartılabilirsiniz.

HERHANGİ BİR ZARARLANMA DURUMUNDA YÜKÜMLÜLÜK/SORUMLULUK KİMDEDİR VE NE YAPILACAKTIR?

Araştırmaya bağlı bir zarar söz konusu olduğunda, bu durumun tedavisi sorumlu araştırıcı tarafından yapılacak, ortaya çıkan masraflar Prof Dr Kemal Türker tarafından karşılanacaktır.

ARAŞTIRMA SÜRESİNCE ÇIKABİLECEK SORUNLAR İÇİN KİMİ ARAMALIYIM?

Uygulama süresi boyunca, zorunlu olarak araştırma dışı ilaç almak durumunda kaldığınızda Sorumlu Araştırıcıyı önceden bilgilendirmek için, araştırma hakkında ek bilgiler almak için ya da çalışma ile ilgili herhangi bir sorun, istenmeyen etki ya da diğer rahatsızlıklarınız için (212) 338 1174 numaralı telefondan Prof. Dr. Kemal Türker ile iletişim kurabilirsiniz.

ÇALIŞMA KAPSAMINDAKİ GİDERLER KARŞILANACAK MIDIR?

Yapılacak her tür tetkik, fizik muayene ve diğer araştırma masrafları size veya güvencesi altında bulunduğunuz resmi ya da özel hiçbir kurum veya kuruluşa ödetilmeyecektir.

ÇALIŞMAYI DESTEKLEYEN KURUM VAR MIDIR?

Şu anda yok ancak ön çalışmalardan sonra destek için müracaat yapılacaktır.

ÇALIŞMAYA KATILMAM NEDENİYLE HERHANGİ BİR ÖDEME YAPILACAK MIDIR?

Yapılacak bu inceleme size veya bağlı bulunduğunuz kuruma hiç bir maddi yük getirmeyeceği gibi, size bu gönüllü katılımınızın karşılığında Prof Dr Kemal Türker'in şahsi kaynaklarından net 50 TL ödenecektir.

ARAŞTIRMAYA KATILMAYI KABUL ETMEMEM VEYA ARAŞTIRMADAN AYRILMAM DURUMUNDA NE YAPMAM GEREKİR?

Bu araştırmada yer almak tamamen sizin isteğinize bağlıdır. Araştırmada yer almayı reddedebilirsiniz ya da herhangi bir aşamada araştırmadan ayrılabilirsiniz; reddetme veya vazgeçme durumunda bile sonraki bakımınız garanti altına alınacaktır. Araştırıcı,

uygulanan araştırma şemasının gereklerini yerine getirmemeniz, çalışma programını aksatmanız vb. nedenlerle isteğiniz dışında ancak bilginiz dâhilinde sizi araştırmadan çıkarabilir. Araştırmanın sonuçları bilimsel amaçla kullanılacaktır; çalışmadan çekilmeniz ya da araştırıcı tarafından çıkarılmanız durumunda, sizle ilgili tıbbi veriler de gerekirse bilimsel amaçla kullanılabilecektir.

KATILMAMA İLİŞKİN BİLGİLER KONUSUNDA GİZLİLİK SAĞLANABİLECEK MİDİR?

Size ait tüm tıbbi ve kimlik bilgileriniz gizli tutulacaktır ve araştırma yayınlansa bile kimlik bilgileriniz verilmeyecektir, ancak araştırmanın izleyicileri, yoklama yapanlar, etik kurullar ve resmi makamlar gerektiğinde tıbbi bilgilerinize ulaşabilir. Siz de istediğinizde kendinize ait tıbbi bilgilere ulaşabilirsiniz

ÇALIŞMAYA KATILMA ONAYI

Yukarıda yer alan ve araştırmaya başlanmadan önce gönüllüye verilmesi gereken bilgileri gösteren beş sayfalık metni okudum ve sözlü olarak dinledim. Aklıma gelen tüm soruları araştırıcıya sordum, yazılı ve sözlü olarak bana yapılan tüm açıklamaları ayrıntılarıyla anlamış bulunmaktayım. Çalışmaya katılmayı isteyip istemediğime karar vermem için bana yeterli zaman tanındı. Bu formu imzalamakla yerel yasaların bana sağladığı hakları kaybetmeyeceğimi biliyorum. Araştırmaya gönüllü olarak katıldığımı, istediğim zaman gerekçeli veya gerekçesiz olarak araştırmadan ayrılabileceğimi ve kendi isteğime bakılmaksızın araştırmacı tarafından araştırma dışı bırakılabileceğimi biliyorum. Bu koşullar altında, bana ait tıbbi bilgilerin gözden geçirilmesi, transfer edilmesi ve işlenmesi konusunda araştırma yürütücüsüne yetki veriyor ve söz konusu araştırmaya katılmayı hiçbir zorlama ve baskı olmaksızın büyük bir gönüllülük içerisinde kabul ediyorum.

Bu formun imzalı ve tarihli bir kopyası bana verildi.

GÖNÜLLÜNÜN		İMZASI
ADI & SOYADI		
ADRESİ		
TEL. & FAKS		
TARİH		

AÇIKLAMALARI YAPAN ARAŞTIRICININ		İMZASI
ADI & SOYADI		
TARİH		
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