Indian J Physiol Pharmacol 2019; 63(1) : 86–93

Medical Education / Method Paper

An Innovative Method to Demonstrate Nerve – Muscle Physiology Experiments Using Finger Muscle Twitch Recording in a Human Subject

Suriya Prakash Muthukrishnan, Sanjay Kumar Patel, Manisha Dubey, Asmita Patil* and Kishore K. Deepak

Department of Physiology,
All India Institute of Medical Sciences,
New Delhi

Abstract

Purpose of the study: Demonstration of nerve-muscle experiments play a vital role for teaching Physiology to undergraduate medical students. In recent days, procuring frogs for the conduction of these experiments have become a major concern. Therefore, we have designed an innovative method to carry out nerve-muscle Physiology experiments in human subjects.

Methodology: We designed a simple and feasible method to demonstrate the effect of increasing strength of stimuli, two successive stimuli and tetanic stimuli on human finger muscle twitch response.

Main findings: Results of four nerve-muscle experiments performed in a human subject using an innovative method has been presented in this article. First, recording of finger muscle twitch by ulnar nerve stimulation. Second, effect of subthreshold, threshold, maximal and supramaximal stimulus with increase in the strength of stimulus. Third, effect of two successive stimuli on finger twitch response. Four, effect of tetanizing stimuli on finger twitch response.

Conclusion: Here we propose a simple innovative practical for effective demonstration of nerve-muscle Physiology experiments using human subject for under-graduate teaching.

Introduction

Nerve muscle experiments have been an integral part of teaching in physiology for the MBBS students as well as the post graduate students. These experiments were performed using sciatic nerve-gastrocnemius preparation (1). In recent days, procuring frogs for the conduction of these experiments have become a major concern.

The skeletal muscle responds to a single brief electrical stimulus by a brief contraction-relaxation cycle. A skeletal muscle belly is composed of large number of muscle fibers and is supplied by a large number of nerve fibers. The motor neuron, together

***Corresponding author :**

Dr. Asmita Patil, Department of Physiology, AIIMS, New Delhi; Email: drasmita@rediffmail.com

(Received on July 1, 2018)

with the muscle fibers innervated by its axon, constitutes a motor unit. The muscle fibers belonging to a motor unit are scattered throughout the muscle belly. Therefore, the gross effect of activation of even one motor unit leads to the contraction of the muscle. If increasingly stronger stimuli are applied to the nerve supplying a skeletal muscle, a greater number of nerve fibers is stimulated resulting in a greater number of active motor units (2). Hence, by increasing the intensity of stimuli, one can recruit more muscle fibers and thereby increase the strength of contraction of the muscle as a whole despite individual muscle fibers displaying an all-or-none response (2-4). If a skeletal muscle is given two successive stimuli of supramaximal intensity, the response to the second stimulus depends upon the time interval between the two stimuli. If a skeletal muscle is repeatedly stimulated at a frequency that it does not relax in between two contractions, it remains in a state of sustained contraction known as tetanus. These concepts of neuromuscular physiology described in this paragraph were demonstrated to the undergraduate students in the Department of Physiology, AIIMS, New Delhi using Amphibian model. In recent days, due to increasing difficulty in procuring frogs, we have designed an alternative method in human subject which is innovative, simple and feasible.

Literature has been searched and it was found that recording of finger muscle twitch has been tried in the past but all of them have used complex protocols which were neither comprehensive nor standardized (5-7). Hence, the current study was conducted in a human subject with an aim to standardize the human nerve muscle experiments for undergraduate teaching.

Materials and Methods

Materials required

The current study was designed to standardize the nerve muscle experiments by human ulnar nerve stimulation for undergraduate teaching. This study was approved by the Institution Ethics Committee, All India Institute of Medical Sciences, New Delhi, India. Result of one healthy human volunteer (aged 27 years, female, right handed) who participated in this study after giving written informed consent has been represented in this current article.

Requirements for this experiment include human subject, digital acquisition system with force transducer and stimulating bar electrode (ADInstruments, Sydney, Australia), and a specially designed forearm brace with little finger stabilization plate (Fig. 1). The forearm brace was designed to improvise the signal noise ratio by minimizing the movement of other muscles supplied by the stimulated nerve. The forearm brace was made using acrylic glass base on which three wooden blocks were fixed to accommodate forearm, wrist joint and hand (Fig. 1). The wooden block for forearm of length 12.5 cm had a broader end (7.5 cm width) for the proximal part of forearm and a narrow end (5 cm width) for the distal part of forearm. The forearm wooden block had a concave curvature to accommodate the convex curvature of the dorsum of forearm. The wooden block for wrist joint (length 2.5 cm, breath 5 cm and height 2.5 cm) was designed to be a mobile block which can fixed to acrylic base of the brace with a screw at any place in between forearm block and hand block. This feature was added to the wooden block for wrist joint to accommodate the difference in the hand length of the subjects. The wooden block for the wrist had a convex curvature to accommodate the curvature of dorsal aspect of wrist joint. The wooden block for the hand of length 5 cm had a narrow end (9 cm) for the proximal hand close to wrist and broader end (12 cm) for the distal hand. The wooden block for the hand had a concave curvature to accommodate the dorsum of hand. The wooden block for hand had another wooden projection fixed on the upper surface of it, which was used to fix 2nd, 3rd and 4th fingers. A layer of foam and synthetic leather was used to cover the wooden blocks. Velcro bands attached with the wooden blocks were used to fix forearm, wrist, hand and fingers. The designing of the brace was inspired from Mosso's ergograph (8). A separate support for little finger was also designed (Fig. 1). Little finger support had provision to fix the proximal phalanx of the little finger and a space for attaching the distal phalanx with the transducer.

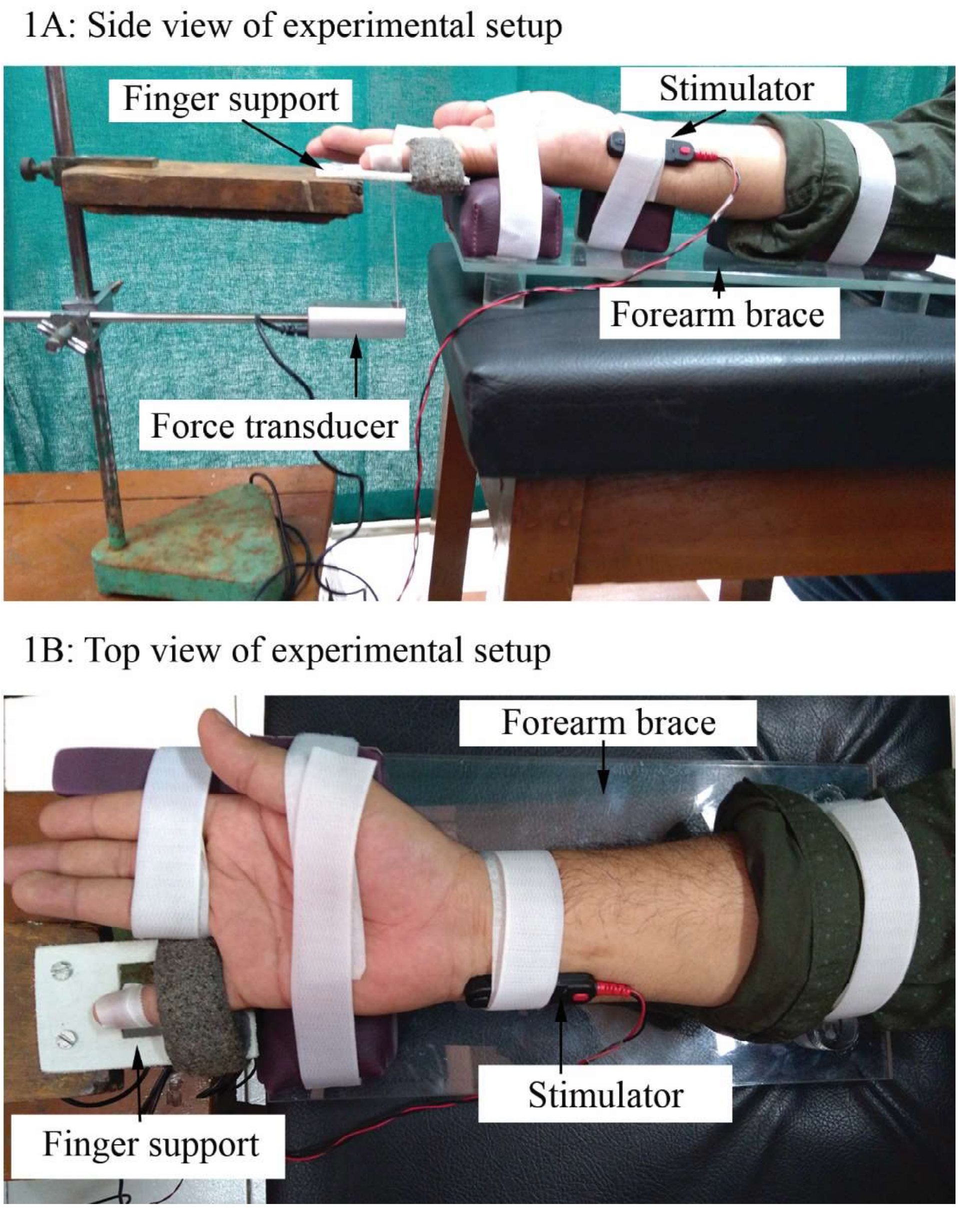

Fig. 1 : Experimental setup for recording human finger muscle twitch response by ulnar nerve stimulation at wrist. Side view (1A) and top view (1B) depicts the application of forearm brace, finger support for little finger, stimulator for ulnar nerve stimulation and force transducer to record flexion at the distal phalanx of little finger.

Experimental Protocol

I) Specific learning objective 1:

To record the finger muscle twitch in the human little finger by ulnar nerve stimulation.

Principle:

Excitation of motor nerve with an adequate stimulus produces contraction of the muscle supplied by it.

Procedure

1. Place the right forearm of the subject on the brace as shown in Fig. 1 to isolate the flexor digitorum profundus inserted at the distal phalanx of the little finger.
2. Tie a loop of thread around the distal phalanx of the little finger and then connect the other end of the thread with the force transducer.
3. Connect the force transducer to “Channel 1” in

the data acquisition box.

4. Clean the stimulation site to be cleaned at medial wrist adjacent to the flexor carpi ulnaris tendon using cotton and spirit (9).

5. Connect the stimulating bar electrode to “Isolated Stimulator” in the data acquisition box.

6. Place the stimulating bar electrode at the stimulation site.

7. Switch on the data acquisition box. Use channel settings, to set “Channel 1” for recording muscle response.

8. Calibrate the Force transducer using known weight.

9. Use stimulator settings, to set “Channel 2” for displaying stimulus time marker.

10. Use “Stimulator Panel” pop-up window to adjust the stimulus parameters. Set the stimulus duration as 0.1 ms.

11. Set the stimulus intensity as 10 mA. Stimulate the ulnar nerve at the stimulation site and record the finger muscle twitch response of the little finger (Fig. 2).

12. Measure the duration of latent period (LP), contraction period (CP), and relaxation period (RP).

II) Specific learning objective 2:

To record the effect of increasing strength of stimuli on the human little finger twitch response.

Principle:

There is considerable variation in the threshold of motor units. Smaller motor units are more excitable than larger counterparts. Increasing the intensity of stimulus over and above the threshold stimulus for a

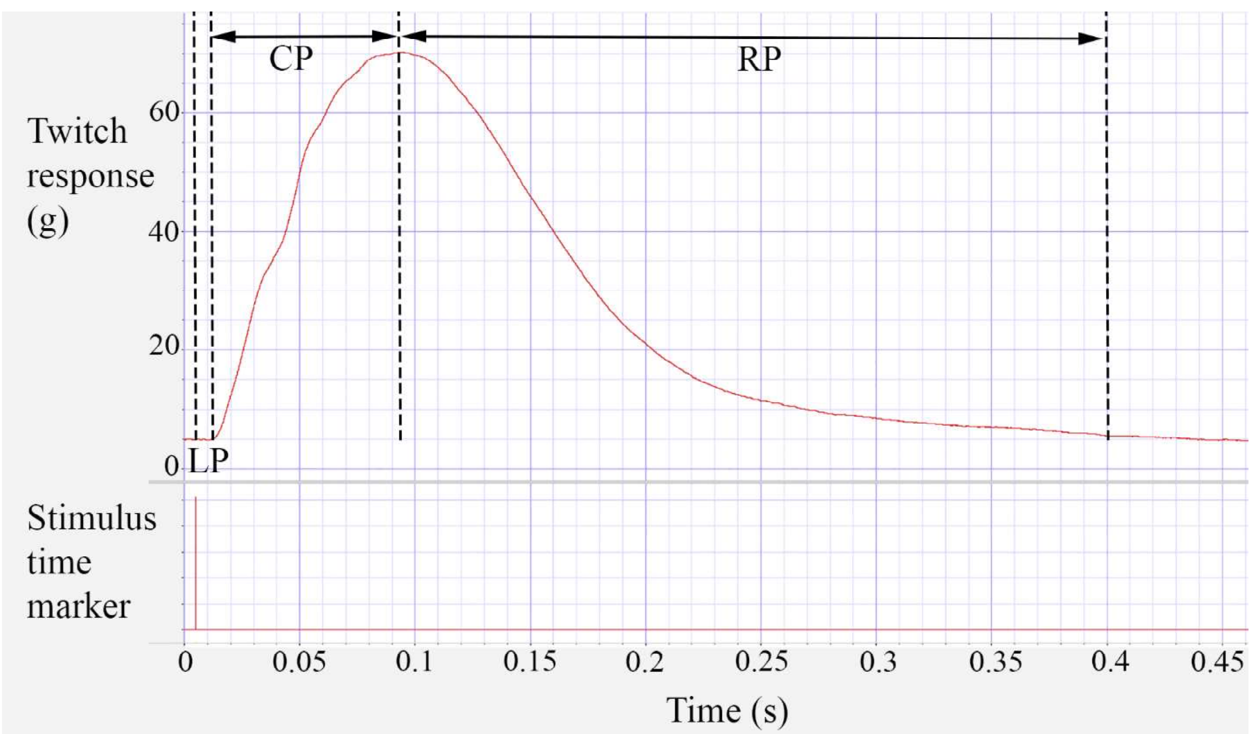

Fig. 2 : Record of muscle twitch response of little finger by ulnar nerve stimulation using a rectangular current pulse of 0.1 ms duration and stimulus intensity of 10 mA. Upper panel depicts the twitch response in Grams. Lower panel shows the time of stimulus application. LP, CP and RP denotes latent period, contraction period and relaxation period. Latent period (LP) is the time interval between the stimulus application to the start of twitch response. Contraction period (CP) is the time interval between the start of twitch to the peak of contraction. Relaxation period (RP) is the time interval between the peak of contraction to the end of relaxation.

muscle excites more number motor units until all the motor units are recruited for the muscle contraction.

Procedure

1. Follow the steps 1-10 of the procedure for the specific learning objective 1.

2. To study the effect of strength of stimuli, set the stimulus intensity to 1 mA and record the response. Step up the stimulus intensity by 1 mA at an interval of 1 minute and record the responses.

3. Minimum stimulus strength at which first recordable twitch can be observed is the threshold stimulus. With increasing stimulus strength, height of contraction should increase and it will reach maximum amplitude (maximal) beyond which there will be no further increase in twitch amplitude by increasing the stimulus strength (supramaximal), Fig. 3.

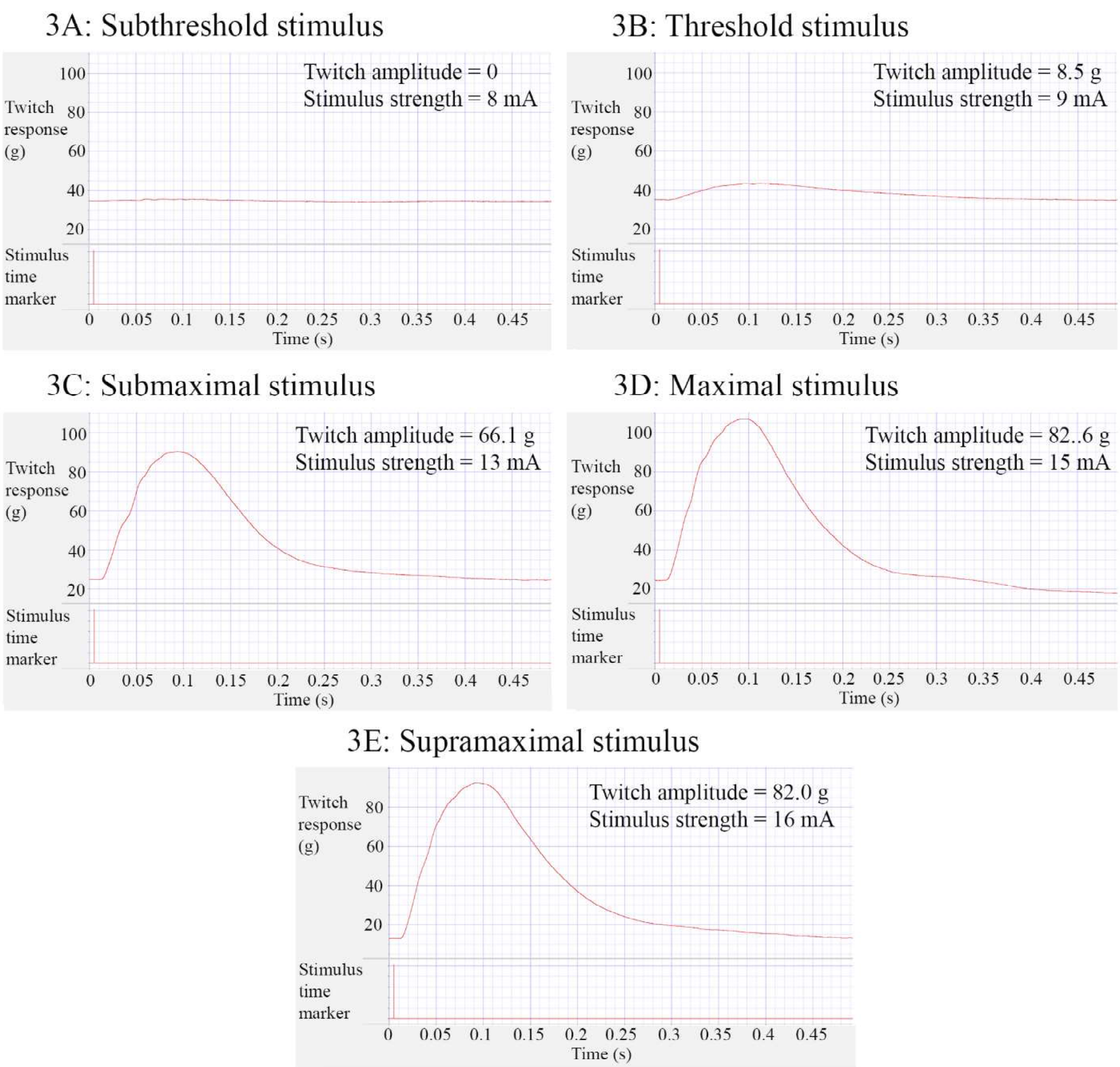

Fig. 3 : Effect of increasing strength of stimulus (a rectangular current pulse of 0.1 ms duration) on finger twitch response. 3A – depicts no twitch response to a stimulus strength (subthreshold stimulus). 3B – depicts appearance of first twitch response with increase in stimulus strength (Threshold stimulus). 3C – depicts more increase in the amplitude of twitch response with increase in stimulus strength above threshold stimulus (submaximal stimulus). 3D – depicts maximal amplitude of twitch response (maximal stimulus). 3E – no further increase in the twitch response with increase in stimulus strength above maximal stimulus (supramaximal strength).

III) Specific learning objective 3:

To record the effect of two successive stimuli on the human little finger twitch response.

Principle:

The effect of two successive stimuli of supramaximal intensity to a skeletal muscle is determined by the time interval between the two stimuli.

Procedure

1. Follow the steps 1-10 of the procedure for the specific learning objective 1.

2. To study the effect of two successive stimuli, set the stimulus intensity to supramaximal intensity. Use stimulator settings, to set the number of stimulations as 2. Adjust the inter-stimulus interval (frequency) in the “Stimulator Panel” such that the second stimulus falls in the following phases.

   I. End of relaxation of the first twitch

   II. During relaxation period of the first twitch

   III. During contraction period of the first twitch

   IV. During latent period of the first twitch

3. Observe the beneficial effect of the two successive stimuli on the twitch response (Fig. 4).

IV) Specific learning objective 4:

To record the effect of tetanic stimuli on the human little finger twitch response.

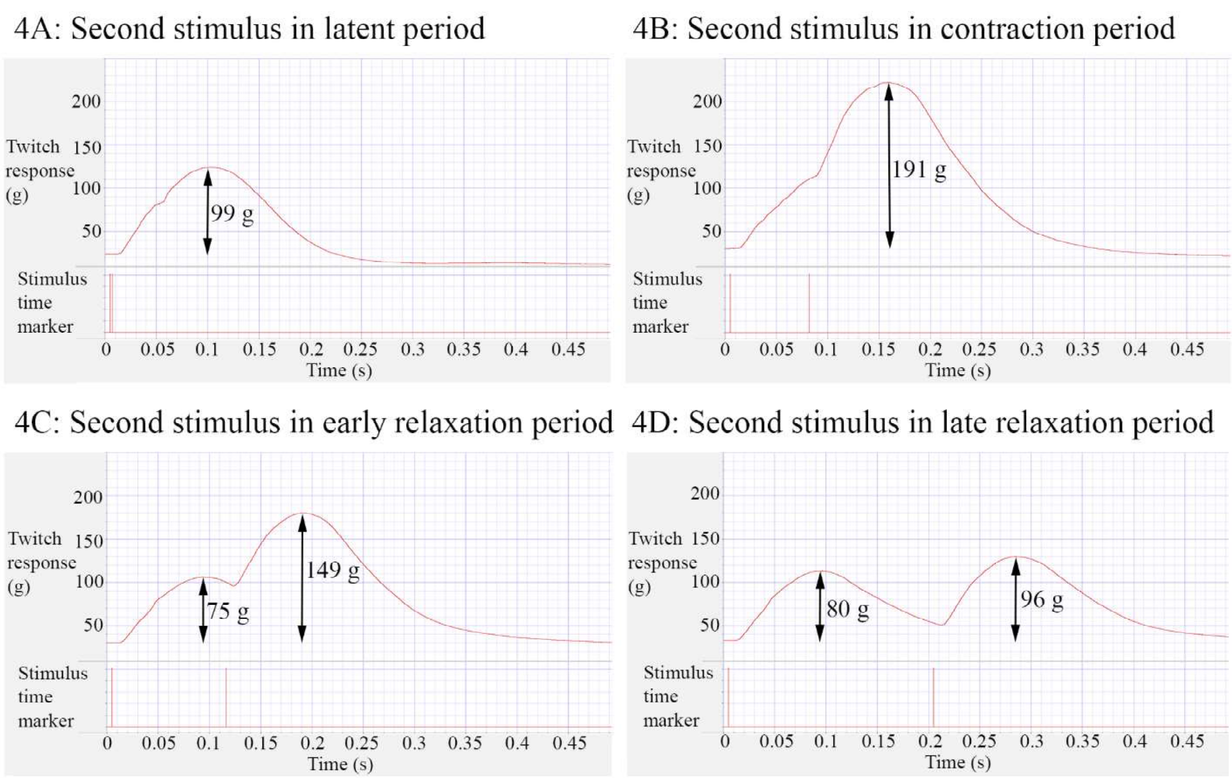

Fig. 4 : Effect of two successive stimuli on finger twitch response. Each stimulus of two successive stimuli was a rectangular current pulse of 0.1 ms duration with supramaximal stimulus intensity of 16 mA. 4A – depicts the no effect on the twitch response by the second stimulus applied during latent period. 4B – depicts increase in the twitch amplitude and increase in the rate of contraction due to the application of second stimulus during contraction period. 4C, 4D – depicts the higher second twitch amplitude than that of first twitch response due to the application of second stimulus during early and late relaxation of first twitch response, respectively.

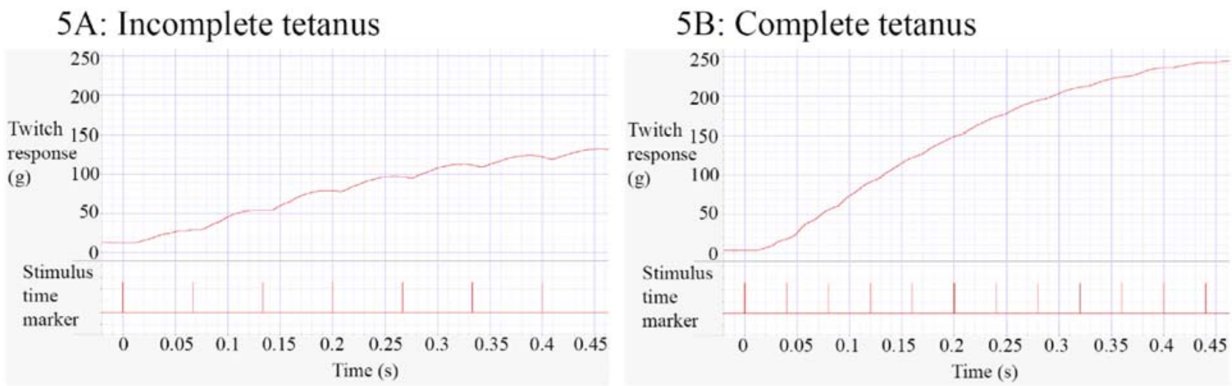

Fig. 5 : Effect of tetanizing stimulus on finger twitch response. Each current pulse in the tetanizing stimulus was a rectangular current pulse of 0.1 ms duration with supramaximal stimulus intensity of 16 mA. 5A depicts incomplete tetanus in which there is a relaxation in between two contractions. 5B depicts complete tetanus in which there is no relaxation between contractions.

Principle:

A state of sustained contraction in response to repeated stimulation with a frequency which does not allow the muscle to relax is known as tetanus.

Procedure

1. Follow the steps 1-10 of the procedure for the specific learning objective 1.

2. To study the effect of tetanic stimuli, set the stimulus intensity to the supramaximal intensity. Measure the duration of latent period (LP), contraction period (CP), and relaxation period (RP). Determine minimum frequency for the genesis of tetanus using the formula :

$$\text{Minimum tetanizing frequency} = \frac{1}{\text{Contraction Period (in seconds)}}$$

3. Use stimulator settings, to set the number of stimulations as infinite (∞). Input the tetanizing frequency calculated in the previous step in the "Stimulator Panel".

4. Observe the effect of tetanic stimuli on the human little finger twitch response (Fig. 5).

Results

The representative records of the finger muscle twitch response and the effect of strength of stimuli, two successive stimuli and tetanic stimuli on finger muscle twitch response are shown in Fig. 2-5.

Finger muscle twitch responses obtained were consistent and reproducible. Average time required for setting up and demonstrating this experiment is expected to be around 1 hour 30 minutes. If this is taken up as a half-day practical of duration 3 hours for the MBBS students, remaining time after the demonstration i.e. 1 hour and 30 minutes can be utilized for the interpretation of the results and discussion of the underlying physiological mechanisms.

Discussion

The present paper proposes an innovative teaching experiment to demonstrate the important concept of motor unit and its recruitment in nerve muscle physiology. The results obtained with the proposed method using human subjects are comparable to the records that we obtained using Amphibian model in our Department. Hence, this could serve as an effective learning tool similar to Amphibian based nerve-muscle experiments for the undergraduate teaching. Moreover, it is not essential to have a digital acquisition system to perform this experiment. The proposed methodology should suit any traditional signal acquisition systems as well.

Using this simple practical demonstration module, students can easily understand the effects of electrical stimuli with varying intensities, two successive stimuli with varying inter-stimulus interval and tetanizing stimuli on the muscle twitch response. Students can correlate their theoretical knowledge with the findings in practical experiments and discuss among themselves about the conceptual aspects of neuromuscular physiology. All this can be achieved without any animals sacrificed. Assessment of student's learning of this demonstration practical can also be done using objectively structured practical examination (OSPE) with question stations based on the graphs obtained during the demonstrations.

Limitation of the proposed method includes the constraint of the forearm brace to study only right-side little finger. There is scope to improvise the design of the brace to study more nerves (radial nerve, medial nerve) and more muscles (other fingers). Further validation of the proposed method is essential and it is currently in progress. The proposed method could potentially be applied to study the drug effect of neuromuscular agents, for dose titration of neuromuscular blockers and for the assessment of neuromuscular disorders.

In conclusion, this proposed innovative methodology can be utilized for effective demonstration of nerve-muscle experiments using human subject for under-graduate teaching in Physiology.

Acknowledgements

We extend our sincere gratitude to the Central Workshop, All India Institute of Medical Sciences, New Delhi for the support in developing the forearm brace used in this study. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Arora R, Berry V. Animal experiments in Medical undergraduate curriculum - assessment of students' views. *Health Administrator* 2005; XVII(1): 63–67.
2. Bigland-Ritchie B, Fugelvand AJ, Thomas CK. Contractile properties of human motor units: is man a cat? *Neuroscientist* 1998; 4: 240–249.
3. Jesunathadas M, Marmon AR, Gibb JM, Enoka RM. Recruitment and derecruitment characteristics of motor units in a hand muscle of young and old adults. *J Appl Physiol* 2010; 108(6): 1659–1667.
4. Doherty TJ, Brown WF. Age-related changes in the twitch contractile properties of human thenar motor units. *J Appl Physiol* 1997; 82(1): 93–101.
5. Westling G, Johansson RS, Thomas CK, Bigland-Ritchie B. Measurement of contractile and electrical properties of single human thenar motor units in response to intraneural motor-axon stimulation. *J Neurophysiol* 1990; 64(4): 1331–1338.
6. Thomas CK, Johansson RS, Westling G, Bigland-Ritchie B. Twitch properties of human thenar motor units measured in response to intraneural motor-axon stimulation. *J Neurophysiol* 1990; 64(4): 1339–46.
7. Häger-Ross CK, Klein CS, Thomas CK. Twitch and tetanic properties of human thenar motor units paralyzed by chronic spinal cord injury. *J Neurophysiol* 2006; 96(1): 165–174.
8. Di Giulio C, Daniele F, Tipton CM. Angelo Mosso and muscular fatigue: 116 years after the first Congress of Physiologists: IUPS commemoration. *Adv Physiol Educ* 2006; 30(2): 51–57.
9. Preston DC, Shapiro BE. Detailed Nerve Conduction Studies. In: Preston DC, Shapiro BE, eds. Electromyography and Neuromuscular Disorders: Clinical-Electrophysiologic Correlations. 3 edition. London, Saunders 2012; 97–124.