

## EXPERIMENTAL ASSESSMENT OF THE EFFECTS OF EPI MUSCULAR MYOFASCIAL FORCE TRANSMISSION IN THE SENSORY LEVEL

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### SUMMARY

The goal was to test the hypothesis that epimuscular myofascial force transmission plays a role in afferent signals generated in muscle sensory organs in passive frog muscles. Ramp-and-hold type of mechanical stimuli (i.e., distal lengthening at a speed of 1 mm/sec to specified stretch amplitude and subsequent keeping of the final position) imposed exclusively on m. gastrocnemius caused sensory activity to be recorded not only from the tibial nerve (innervating m. gastrocnemius and synergistic m. tibialis posticus) but also from the common peroneal nerve (innervating antagonistic m. peroneus, m. tibialis anticus, m. extensor cruris brevis and m. tibialis anticus brevis), despite the fact that all non-targeted muscles were kept isometric. Such finding explained by epimuscular myofascial force transmission confirms our hypothesis and may have major implications for our understanding of the functioning of muscular mechanoreceptors.

### INTRODUCTION

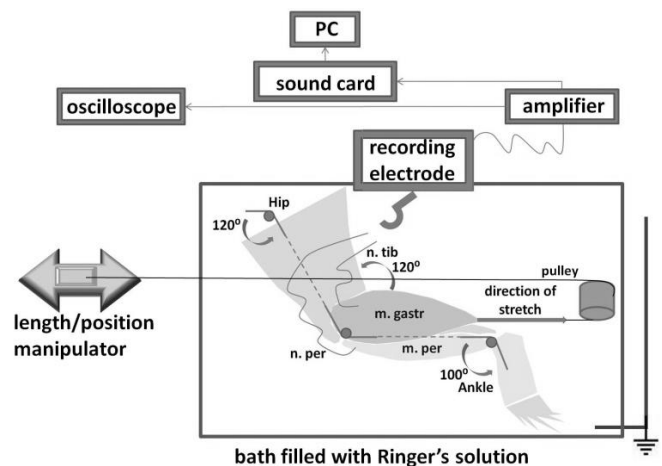
Epimuscular myofascial force transmission has been shown to have major effects on muscular mechanics including substantial proximo-distal force differences and distribution of lengths of sarcomeres located within muscle fibers [for recent reviews of the key concepts see 1, 2]. Moreover, such force transmission was shown to occur not only among synergistic muscles but also among the antagonistic muscles within an entire limb [e.g., 3]. Based on these findings we hypothesized that epimuscular myofascial force transmission plays a role in afferent signals generated in muscle sensory organs. Our goal was to test this hypothesis.

### METHODS

Surgical and experimental procedures were in strict agreement with the guidelines and regulations concerning animal welfare and experimentation set forth by Turkish law and were approved by the Animal Experiments Committee at Boğaziçi University, Istanbul. 24 frogs were used (*Rana ridibunda*, either sex, body mass 24 to 51 grams). Frogs were cold-anesthetized at -18°C for approximately 5 minutes and pithed promptly afterward. Subsequent to removal of the skin, the leg was fixed using clamps and bone pins, such that the hip, knee and ankle joint angles were kept at 120°, 120° and 100°, respectively. For this reference configuration, the position of Achilles tendon was marked prior to performing tenotomy as distally as possible. The tendon was then tied securely to a length/position manipulator (Figure 1). The fasciae between

m. sartorius and m. adductor magnus of the upper leg were dissected in order to expose the sciatic nerve. Tibial and peroneal branches of the sciatic nerve were cut close to the bifurcation. Individual branches were identified by applying brief electrical stimulation to each branch and observing the responding muscle group. The nerve branches were then placed on a plastic stratum. Using a dissection microscope, each of the nerve branches were teased apart progressively until the nerve fiber at hand is as thin as can be achieved. The dissected nerves were placed on a tungsten hook electrode.

Ramp-and-hold type of mechanical stimuli was used to elicit sensory responses from the passive lower leg muscles: (1) m. gastrocnemius was lengthened distally by moving the position/length manipulator: muscle-tendon complex was stretched at a speed of 1 mm/sec. For each individual sensory unit selected, five stretch amplitudes were used during the ramp phase: 1 mm, 2 mm, 3 mm, 4 mm and 5 mm. (2) Following stretch, the manipulator was kept at its final position imposing the hold phase.



**Figure 1:** Schematics of the experimental set-up

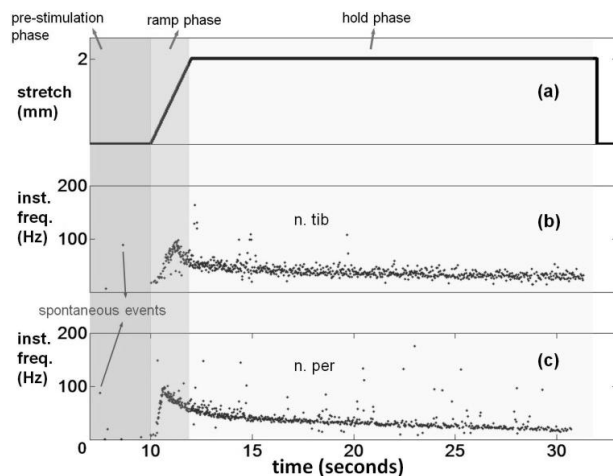
The signal from dissected nerves was recorded: the electrode signal was amplified with a custom build amplifier (x10 000 gain, 50 Hz notch filter, 10 Hz high pass filter, 10 kHz low pass filter), and digitized by an external data-acquisition card (Soundblaster Digital Music SX, 24 bits sampling rate: 44.1 kHz; Creative Labs, Singapore). Three phases were distinguished: (1) signals during 10 seconds prior to stretch (*pre-stimulus phase*), (2) the *ramp phase* and (3) 20 seconds of *hold phase* characterizing receptor adaptation. Separate

recordings were collected from the tibial nerve (n=12), innervating m. gastrocnemius and m. tibialis posticus, the latter being restrained (isometric); and from the common peroneal nerve (n=12), innervating m. peroneus, m. tibialis anticus, m. extensor cruris brevis and m. tibialis anticus brevis; all of which are restrained muscles.

A custom code was used to obtain times of the individual spikes coming from a single sensory unit: an adjustable window (made up of two straight lines representing voltage values flanking the peaks of spikes from above and below) was used to select individual spikes of a unit. For each stretch amplitude, inter-spike time intervals were used to calculate instantaneous firing frequency, as well as mean spike frequency for the three phases. Two-way ANOVA for repeated measures (factors: mechanical stimulus phase; i.e., pre-stimulus, ramp, and hold respectively, and stretch amplitude) was performed for tibial as well as peroneal nerve recordings. If significant main effects were found, Bonferroni post-hoc tests were performed to further locate significant differences. Mean phase firing frequency differences were considered significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

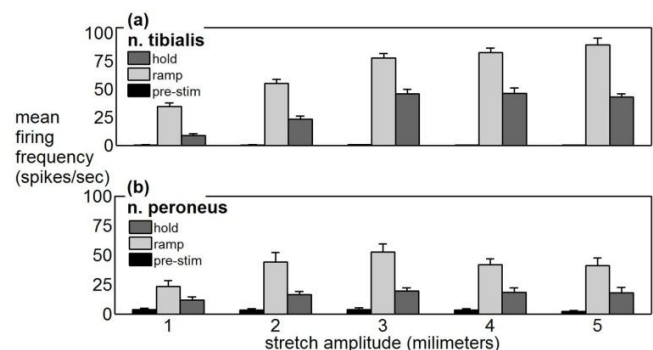
Characteristic features of recordings from both nerve branches (Figure 2) are similar: (i) for the pre-stimulus phase, sensory activity is almost non-existent except for minor spontaneous firing. (ii) In contrast, instantaneous frequency charts recorded from both nerve branches show a burst of mechano-receptor activity during the ramp phase, and (iii) receptor adaptation to maintained stretch during the hold phase.



**Figure 2:** Typical example of instantaneous firing frequency as a function of time. Mechanical stimulus of m. gastrocnemius (a) and recordings from tibial (b) and peroneal nerve (c).

Mean phase firing frequencies are shown in Figure 3. For recordings from both nerve branches, ANOVA showed significant main effects of stretch amplitude and mechanical stimulus phase on mean phase frequency, as well as a significant interaction between these factors. (I) Tibial nerve

activity Note that this branch carries the sensory signals from m. gastrocnemius (passively lengthened) and m. tibialis posticus (restrained). Maximal pre-stimulus, ramp and hold phase mean firing frequency values measured were  $0.45 \pm 0.33$ ,  $89.63 \pm 5.85$  and  $46.30 \pm 4.74$  spikes/second, respectively. For both ramp and hold phases, post hoc tests located significant differences in mean firing frequencies for comparisons between stretch amplitude = 1 mm and all higher stretch amplitudes and for comparisons between stretch amplitude = 2 mm and the remainder higher stretch amplitudes. (II) Common peroneal nerve activity It should be noted that this nerve transmits sensory information from no other muscles than the restrained extensor muscles with the likelihood of sensory information also from associated extramuscular tissues. Maximal pre-stimulus, ramp and hold phase mean firing frequency values measured were  $3.76 \pm 1.27$ ,  $52.39 \pm 6.91$  and  $19.39 \pm 2.70$  spikes/second, respectively. For the ramp phase, post hoc tests located significant differences in mean firing frequencies exclusively for comparisons between stretch amplitude = 1 mm and all higher stretch amplitudes. However, differences between hold phase mean firing frequency values were not significant for any stretch amplitude (post hoc).



**Figure 3:** Mean phase firing frequencies plotted as a function of stretch amplitude. Tibial nerve branch (a) and Peroneal nerve branch (b).

## CONCLUSIONS

These novel findings show that passive lengthening of m. gastrocnemius elicits sensory response not only from the mechanoreceptors of targeted muscle but also from those of restrained, passive synergistic as well as antagonistic muscles. Regarding the latter, firing of e.g., muscle spindles is conceivable in conditions with no global length changes to the muscle fibers but due to occurrence of local deformations caused by epimuscular myofascial force transmission. The present results confirm our hypothesis and are likely to have major implications for our understanding of the functioning of muscular mechanoreceptors.

## REFERENCES

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