Fibroblasts Regulate Local Strains That May Modulate Neuron Activity for Injurious Stretch in a Collagen Gel Simulating a Ligament

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INTRODUCTION

When neural tissue is stretched, the neurons can undergo injury and impaired function [1,2]. Nerve fibers and fibroblasts are mechanosensitive [3,4]. Both cell types not only react to direct loading but can also initiate responses when exposed to indirect loading, which can occur when their surrounding extracellular matrix components, like collagen, are distorted [5,6].

As a result of mechanical stimuli, these cells can release chemical factors that regulate neuronal activity [7,8]. Although neuronal function and activity have been studied in the context of macroscopic stretch [8,9], their relationship to local strains and the effects of fibroblasts, which are known to alter the local mechanical environment[10] is less clear.

Study Objective: The goal of this study is test the hypothesis that the presence of fibroblasts in a co-culture system changes local strains and modulates neuronal activity.

METHODS

Sample Preparation: Collagen gels (2mg/mL) were fabricated with fluorescent microbeads (40nm dia.; 608nm emission; ThermoFisher). Two sets of gels were allowed to self-assemble [11]: with fibroblast-like-synoviocytes ($5x10^4$ cells/mL) (n=2) and without (n=3). Rat embryo cortical neurons were seeded on gels and cultured as previously described [12].

Testing: At DIV2, neurons were transduced with an AAV expressing GCaMP6f in the feeding media, which causes neurons to fluoresce at 488nm with calcium influx [13]; at DIV6, collagen (150µL) was added. At DIV9, gels were placed on a stretching device and imaged under a Leica inverted spinning disc confocal microscope (Fig. 1). Gels were stretched to 3 different macroscopic strains: physiologic (8-10%), painful (20-30%), and supraphysiologic stretch (>30%) [14,15]. Image series (20fps for 60s) were captured before stretch in the unloaded condition and at each macroscopic strain level.

Analysis: ImageJ was used to separate the image stacks by color in order to visualize fluorescent beads (red channel) and neurons (green channel). Displacements of the fluorescent beads were tracked using ProAnalyst and 4-node elements were created for each gel (Fig. 1). The maximum principal strain (MPS) of each element at each condition was calculated relative to the unloaded condition using LS-DYNA. The average elemental MPS across gels was compared using repeated measures ANOVA. FluoroSNAPP analyzed the number of calcium events for each neuron [16] in each element and the average number of events per active neuron was compared between groups by repeated measures ANOVA.
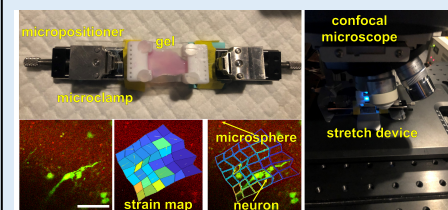

Figure 1.
Gels were stretched and imaged, and images analyzed to calculate local strains and neuronal activity (scale bar = 50µm)

Statistical Analyses: The peak tensile and compressive forces, and stiffness were compared between Cycle1 and Cycle20 using paired t-tests. In addition, loads at FR0 and End were also compared to the load at the peak of the ramp by using separate paired t-tests. CV values were similarly compared between reference and each of the events (Cycle1, Cycle20, Ramp, FR0, End) using separate paired t-tests.

FUNDING

RESULTS

Average Elemental MPS:

- In the presence of fibroblasts, local elemental MPS is not different for low stretches, either at physiological (12±18%) or painful (15±23%) stretch, compared to the local strains in non-seeded collagen gels (9±8%; 20±21%) (Fig. 2). At supraphysiological stretch, elemental MPS is significantly lower (p<0.01) with fibroblasts (4±5%) than without (30±25%) fibroblasts.

Average Activity Per Neuron

- There is a similar difference in the neuron activity at supraphysiological stretch (Fig. 2), with neurons in non-seeded gels having 5-fold more activity (5±3) compared to neurons in fibroblast-seeded gels (1±1).
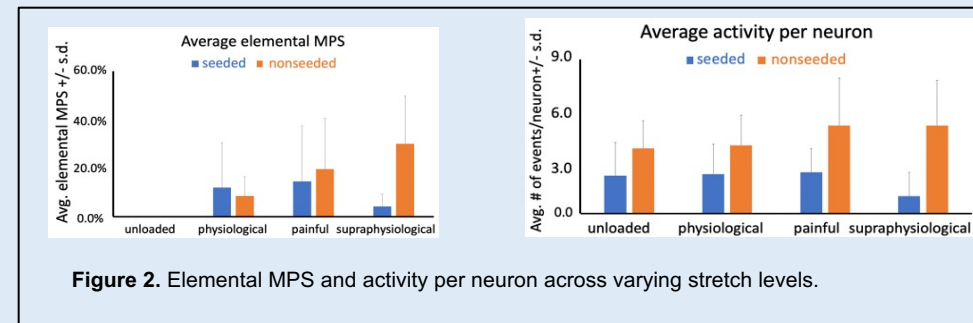

Figure 2. Elemental MPS and activity per neuron across varying stretch levels.

DISCUSSION

While no significant differences in transient neuronal activity were found, fibroblasts can release MMPs over 24h [11], suggesting effects on neuronal activity may be delayed.

Fibroblasts suppress elemental MPS at supraphysiologic stretch, which also decrease neuron activity.

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