Kelara Samuel Abstract

Thoracic Intraspinal microstimulation (ISMS) evokes frequency dependent activation of respiratory efferents

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Cervical spinal cord injury can impair breathing by preventing brainstem generated respiratory activity from reaching motor pools in the spinal cord which drive respiratory pump muscles. We have previously demonstrated that high frequency intraspinal thoracic spinal cord stimulation can produce respiratory activity independent of centrally generated signals. While this high frequency stimulation (300Hz) results in “asynchronous” activation of spinal respiratory outputs, studies from our laboratory have demonstrated that direct activation of respiratory motor nuclei produce entrained activation. As we investigate the spinal circuitry involved in activating respiratory efforts with thoracic stimulation, the response to lower frequencies has not been examined. We hypothesize that targeted intraspinal microsimulation (ISMS) within the thoracic spinal cord at lower frequencies (50-200Hz) will result in entrained activation of both the phrenic and thoracic respiratory outputs. To investigate this, we employed the *in situ* rat preparation to characterize stimulus response patterns using bipolar stimulating electrodes in the ventrolateral thoracic spinal cord (T2-6). Our initial results demonstrated entrained activation at lower frequencies while higher frequencies showed asynchronous activity on respiratory efferents. We conclude that high frequency thoracic ISMS efficiently and reliably activates both the phrenic and thoracic efferents, but activation patterns are dependent on stimulus train frequency. We propose that the asynchronous pattern previously observed with high frequency ISMS outpaces the intrinsic firing ability of the motoneurons, giving the impression of physiological activation patterns. Ongoing studies continue to focus on mechanisms of activation of respiratory neural circuits activated by thoracic ISMS.

Santiago Carrasquilla Abstract

Application of Traction Force Microscopy in Pharmacogenetic Studies Using Patient-Specific Studies Using Induced Pluripotent Stem Cells

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Introduction: Since the introduction of induced pluripotent stem cells (iPSC), the interest for patient specific models in developmental biology, regenerative medicine and drug screening has increased [1]. By better understanding the biology of iPSC derived cells more accurate patient specific response models can be devised for drug screening. We assessed the strain energy and traction force of iPSC derived smooth muscle cells (iVSMC) by Traction Force Microscopy (TFM) [2].

Materials and Methods: A two step process was implemented to place 0.52 µm fluorescent beads on the surface a polyacrylamide (PAA) gel. The bottom layer of the PAA gel was attached to a silicone sheet using benzophenone [3], and then a second thin layer of PAA gel with embedded fluorescent beads was polymerized on top. The gel was functionalized with sulfo-SANPAH and coated with collagen. In order to have sparse single cell density, Human Umbilical Artery Smooth Muscle Cells (HUASMC) and three patient specific iVSMC lines (A, B, and C) were seeded at ~500 cells/cm2 . The cell were then exposed to with 10ng/ml of endothelin-1 (ET1) for 30 minutes. Images of the beads and single cells were taken immediately after cell seeding (basal), after drug exposure (ET1), and after application of trypsin (null). Bead displacements were calculated using PIVlab [4] and TFM calculations [2] were performed to determine the strain energy of contraction for each cell.

Results and Discussion: Basal and ET1 induced contraction of HUASMC (n=21), Patient A (n=13), Patient B (n=20) and Patient C (n=20) were statistically different from one another (Fig. 1, ANOVA $p< 0.05$). Figure 1. [Above] Sample phase image of cell on gel and respective traction force diagram. Scale bar is 50 µm. [Right] Strain energy fold change in contractility (ET1/Basal) for iVSMC. HUASMC A B C

Conclusions: Basal behavior and endothelin-1 response of hypertensive iVSMC is drastically different from non-hypertensive primary HUASMC. Furthermore, we see patient-derived cells respond differently to ET1. Understanding patient specific response to small and large molecules can advance precision medicine by increasing reliability and lowering treatment costs. Ongoing and future experiments will characterize the response of these and additional patient lines to other physiological modulators, such as angiotensin-II, and hypertensive drugs, such as beta-blockers.

References: [1] M Mercola et al. Circ Res 2013, 112:534-548; [2] C Mierke et al. Biophys J 2007, 94(2):661-670; [3] CS Simmons et al. Lab Chip 2013, 13(4):646–649; [4] W Thielicke et al. J Open Res Software 2014, 2.