SUBSTANCE P AND VASOACTIVE INTESTINAL PEPTIDE ENHANCE OVERALL CELLULAR PROLIFERATION DURING EARLY MCL HEALING

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Introduction
Neuropeptides such as substance P (SP) and vasoactive intestinal peptide (VIP) have been shown to influence immune and inflammatory responses to wound healing. Tissue types studied thus far have included skin, lung, prostate, and intervertebral discs.

Previous data from our lab has shown that local delivery of each SP and VIP has significantly increased the mechanical strength of healing medial collateral ligaments (MCL) in an in vivo animal model. There are several possible factors that may contribute to this result, one of which may be that SP and VIP increase cellular proliferation following ligament injury. We hypothesize that local delivery of SP or VIP to a healing MCL will significantly increase cellular proliferation following injury.

Methods
An animal model for the local delivery of neuropeptides has been developed and tested. SP (75pg/100µl) or VIP (100pg/100µl) with BrdU (0.2mg/µl) infused mini-osmotic pumps (Alzet Corp) were implanted subcutaneously into the low back of 12 rats (N=3 for each group; 220-250 grams). MCL rupture was performed on both legs. To one leg a small catheter running from the pump to an intramuscular space above the MCL was secured. Pumps delivered the neuropeptide and BrdU at a rate of 0.25µl/hr continuously for two weeks, at which time rats were euthanized and ligaments were harvested and flash frozen in optimal cutting temperature solution and liquid nitrogen. The contralateral side served as a healing control. Six sham animals received no MCL rupture.

Immunohistochemical analysis was performed and BrdU staining quantified using 20X microscope and Image J (NIH).

Results
Within minutes of surgical intervention, all rats recovered and had normal movement and behavior (grooming, feeding, etc.). MCLs supplemented with SP and VIP each showed significant increases in BrdU labeling than contralateral controls (594±38.4 vs. 220±26.6 cells, paired Student’s t-test p<0.001; 376±56.1 vs. 154±24.5, paired Student’s t-test p=0.016 cells; Figure 1).

Discussion
This study supports our hypothesis that local delivery of SP and VIP each significantly increase cellular proliferation of rat MCLs following surgical transaction. A unique aspect to this experiment was that cell counts represented BrdU labeling throughout the entire 14 day experimental period. In contrast to using one BrdU injection, continuous labeling through the mini-osmotic pumps enabled us to characterize DNA replication throughout the entire healing process. These data agree with previous wound healing studies that have shown SP and VIP to influence cellular proliferation. This study then suggests that the substantial increase in ligament healing strength is related to the cell proliferation associated with neuropeptide treatment.

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Figure 1: Local delivery of SP and VIP to healing MCLs with continuous BrdU labeling over a 14-day period (average cell counts with +/-SE bars). Neuropeptide treated healing ligaments exhibited significantly more BrdU labeling when compared with controls (p<0.001 and p=0.016 for SP and VIP, respectively).

References: