Introduction: The repair of cartilage is a central issue in orthopedic care. Because cartilage lacks regenerative ability, treatment for cartilage disease is primarily analgesic or surgical. Neuropeptides play a role in the proliferative and reparative processes of many tissue types, including fibroblasts, which arise from the same progenitor cells as chondrocytes (1). There is also some evidence that neuropeptides have a direct effect on articular chondrocytes (2,3). This paper aims to investigate the effect of four candidate neuropeptides on articular chondrocyte proliferation.

Materials and Methods: Bovine chondrocytes were cultivated in monolayer culture in media alone or media containing one of four neuropeptides: neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP), substance P (SP), or VIP.

Available cartilage from the articular surface of the femoral trochlea, femoral condyles, and patella of freshly slaughtered veal knees (n=8) was enzymatically digested overnight in Dulbecco’s Modified Eagles Media (DMEM) containing 0.5 mg/mL hyaluronidase and collagenase. These chondrocytes were then plated at 1x10^5 cells/mL in DMEM complete media with 5% FCS and PCN/Strep. Cells were plated using 100 μL of the cell suspension in a 96 well, opaque-walled, tissue culture plate with 6 replicates for proliferation analysis. After 24 hour incubation, media was exchanged with new DMEM complete media with the appropriate treatment. The treatment groups consisted of a control (media only), a positive control (media + 100 ng/mL IGF-1) and the treatment group (media with neuropeptide). Treatments included three concentrations of each neuropeptide. The SP concentrations were 5, 50, and 500 μg/mL, the CGRP concentrations were 10, 100, and 1000 ng/mL, and the NPY concentrations were 0.05, 0.5, and 5 μg/mL. Finally, the VIP concentrations were 100, 1000, and 10,000 ng/mL. Media was exchanged every two days with new DMEM plus treatment. The cultures were carried out to day 8. Proliferation assays, assessed with the Promega Cell Titer Glo luminescence assay, were run every two days. Analysis was performed using a split-plot ANOVA and pairwise t-tests.

Results: CGRP showed no significant effect on cartilage proliferation at all concentrations at all time points. SP showed a stimulatory effect on proliferation on all days. This increase was statistically significant for SP at 5 and 50 μg/mL on days 2-6 (see figure 1). NPY and VIP showed a dose dependent suppressive effect on chondrocyte proliferation. This effect was greatest for both VIP and NPY at their highest concentrations and was significant at all time points (see figure 2).

Discussion: Neuropeptides play an essential role in the homeostasis of many tissue types yet there is limited evidence of their interaction with cartilage. CGRP and SP have been previously shown to exert direct effects on articular chondrocytes (2,3). Our current study supports these findings that neuropeptides have direct metabolic effects on articular chondrocytes. SP showed a reliable stimulation of chondrocyte proliferation in this study. This is consistent with findings that SP caused a stimulatory effect on cartilage disc cell proliferation (4). SP may hold some potential to help orchestrate biologic repair of articular cartilage injury. NPY and VIP showed dose dependent depressant effects on cartilage growth. Neuropeptides have been strongly implicated in inflammatory arthritis (5) and the current findings raise the question whether the direct effect of NPY and VIP on chondrocytes may play a role in this process. Improved understanding of the interaction between the peripheral nervous system and articular cartilage may help in clarifying the pathophysiology of cartilage degeneration. In addition, the knowledge that these neuropeptides have a direct effect on chondrocytes growth opens the possibility for manipulating these bioactive factors to help prevent cartilage degeneration or direct cartilage repair.