Development Of a Reproducible In Vivo Rat Model for Investigating Ligamentum Flavum Hypertrophy and Pain-Related Behaviors

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INTRODUCTION: Lower back pain (LBP) is the leading cause of years-lost-to-disability, costing an estimated 0.1-2% of GDP in industrialized countries, with lumbar spinal stenosis (LSS) as a primary contributor.[1,2] A hallmark of LSS is ligamentum flavum hypertrophy (LFH), which results in spinal canal narrowing and neurogenic pain. The high socio-economic costs of LFH are associated with reductions in professional and recreational activity and anti-inflammatory/analgesic therapies. However, in vivo models for studying LFH and associated functional impairments remain limited.[3] This study introduces a novel, reproducible rat model of LFH induced via biomechanical spinal instability, designed to simulate clinical pathology and assess rehabilitation-relevant pain behaviors. We hypothesize that inducing spinal instability in a rat model will lead to LFH and functional deficits, including altered pain-related behaviors and reduced mobility, mimicking clinical features of lumbar spinal stenosis, and providing a platform for evaluating rehabilitative interventions.

METHODS: Sixteen adult male Fischer 344 rats were randomized into instability (n=8) and sham (n=8) cohorts. The instability group underwent surgical bilateral pars defect creation at L4-L5, inducing mechanical instability without extensive soft tissue disruption, while sham rats had paraspinal musculature retracted but no bony resection. Behavioral assessments (distance, velocity, grooming and rearing) were performed at 2, 4, 6 and 8 weeks post-surgery. At 8 weeks, animals were euthanized for imaging and histology. All behavioral tests were performed in a black 50 × 50 cm open-field enclosure, as previously described. [4] Videos of rat behavior were captured using a Basler GigE IR camera set in bird's eye position, while motion and behavior were recorded and analyzed using Ethovision XT Automated Behavior Recognition software (Noldus Information Technology). Isolated spines were harvested and fixed for 24 hours before CT and MRI imaging. Afterwards, samples were decalcified in Immunocal™ (StatLab) for 14 days before paraffin embedding. Ten micro sections of samples were then stained by Hematoxylin and Eosin for tissue morphology and cellularity and Verhaoff's elastin stain to detect changes in elastin, a key marker of LFH. Histonorphologic analysis was performed with ImageJ. Statistical significance was set at $p < 0.05$.

RESULTS: At 8 weeks. rats with induced instability exhibited trends toward increased grooming (44.1 ± 7.5 seconds more) and decreased rearing (17.2 ± 2.4 seconds less)—behaviors indicative of discomfort and functional limitation—though not statistically significant ($p > 0.05$) (Fig 1A&B). MRI revealed significant focal canal stenosis at the surgical level (mean canal area: 2.04 mm² vs. 5.99 mm² at non-surgical levels, $p < 0.05$). MicroCT confirmed pars defects with variable bone healing, although there was variation in the level at which surgery was performed. Histology showed ligamentum flavum disorganization and hypercellularity (Fig 2A&C) of 36% increase in thickness (mean quartile thickness of 97 mm vs 62 mm at the surgical level , $p < 0.05$) (Fig. 1C) and localized loss of elastin (Fig 2B&D) quantified as 2.3-fold reduction (n=1) in elastin content in the instability group, consistent with LFH pathogenesis.

DISCUSSION: The hypothesis of this study was partially supported: spinal instability induced stenosis and LFH; however, spine instability (and LFH) did not lead to significant changes in functional deficits. The degree of hypertrophy reported here is lower than previous studies and may reflect the absence of specific biomechanic conditions used in this study that otherwise induce hyper-flexion and hyper-extension and increase hypertrophy. Under these reported experimental conditions, the 8-week time point may also represent an early stage of disease progression, where histology shows significant but not transformative regional and heterogenous hypercellularity and elastin loss. The behavioral analysis at 8 weeks, although not significant, may indicate an emerging change in behavior by rats in the instability group. To clarify these hypotheses, additional cohorts using longer time-points and/or methods to induce hyper-extension/flexion must be carried out with the addition of with transcriptomic and proteomic analyses.

SIGNIFICANCE/CLINICAL RELEVANCE: This is the first reported *in vivo* rat spine instability model to include behavioral assessments aimed at studying the pathogenesis of LFH resulting in LBP. The development of this simple spine instability model will aid in identifying mechanisms of LFH and serve as a platform to test therapeutics.

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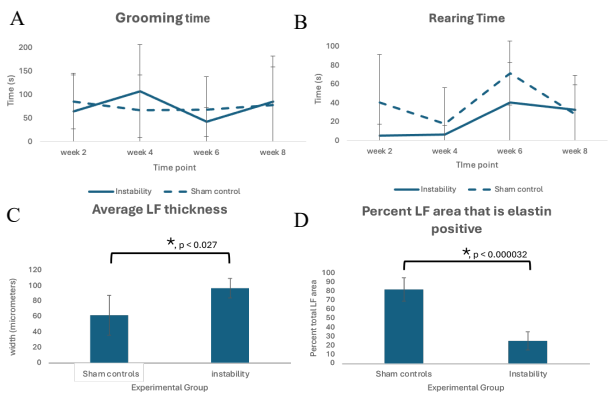

Fig. 1: (A&B) Quantitative assessment of LF thickness and positive elastin stain and (C&D) behavioral outcomes in instability and sham control groups

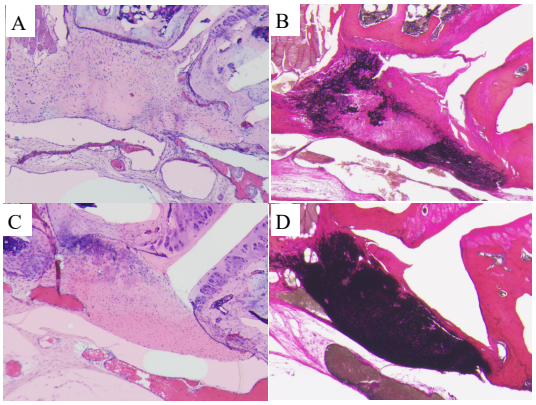

Fig. 2: Histological staining of the LF in the (A&B) instability and (C&D(sham surgical groups by (A&C) H&E and (B&D) Verhaoeff's Elastin Stain