

Post-traumatic osteoarthritis model in aged rats induces changes in tactile sensitivity and brain and joint remodeling

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INTRODUCTION: Osteoarthritis (OA) is a debilitating and painful joint disorder with a significant impact on patient's lives. Preclinical models provide useful details regarding joint-level remodeling and pain-related behaviors with disease progression, but effects beyond the joint are poorly understood. One example of this limited understanding is the unknown role of the nervous system in OA progression. In patients with OA, brain remodeling of pain processing regions has been observed. However, specific mechanisms through which this brain remodeling occurs in OA are unknown, and brain changes in preclinical models of OA are weakly characterized. To address this limitation, the current study investigates joint remodeling, pain-related behavior, and brain remodeling in a surgical rodent model of post-traumatic osteoarthritis. Additionally, aging is an important confounding factor in brain remodeling due to its effect on neuroplasticity. Therefore, we used aged animals to account for the aging component. Here, we leverage commonly utilized techniques such as histological analysis of the knee joint and longitudinal testing of tactile sensitivity behavior while adding in magnetic resonance imaging (MRI). This design allows us to evaluate both disease progression and the effects of early (6 weeks) and late stage (14 weeks) OA on brain remodeling in aged rats.

METHODS: Male and female Fischer 344 rats – 18-19 months of age at time of surgery – received either a skin incision (n= 6 male and n= 10 female) or medial meniscus transection plus medial collateral ligament transection (MMT+MCLT) (n= 5 male and n= 11 female) surgery. Tactile sensitivity behavioral testing was performed before surgery and 4-, 8-, 12-, and 16- weeks post-surgery. MRI scans were collected on a 11 T/40 cm horizontal magnet (Bruker) prior to surgery and 6- and 14- weeks post-surgery. Structural MRI's were spatially registered to the Waxholm brain atlas. Using the predefined atlas, functional connectivity was calculated by taking the correlation of each brain region's time series signal with every other regions' signal. Functional connectivity analysis focused on regions with relevant physiological significance pain processing including regions involved with sensory input, sensory perception, nociception, and the emotional aspect of pain. Tactile sensitivity and functional connectivity data were analyzed using linear mixed effects models where surgery group and imaging timepoint were treated as fixed effects, and animal was treated as a random effect. Separate analyses were performed for male and female animals. When indicated by the analysis of variance, specific differences between fixed effects were assessed via Tukey's post-hoc comparisons of least squared means. After 18 weeks, animals were euthanized, and histological analysis was performed to assess joint changes. Frontal sections (10 μm) representing the loading region on the medial tibial plateau were stained with safranin-O and fast green. Probabilistic heat maps were generated for each group to visualize joint level remodeling in the cartilage and subchondral bone. These heat maps were created by co-registering the binary images of the histological regions of interest – such as subchondral bone and cartilage –, taking the sum of the stacked images, and then dividing by the number of images in the stack.

RESULTS: In MMT+MCLT animals, there was a trend of decreased 50% withdrawal threshold compared to skin incision controls at 4-,8-,12-, and 16- weeks post-surgery (Fig. 1). Functional connectivity changes are visualized in Fig. 2A and 2E for females and males, respectively. For females, there was higher functional connectivity for the MMT+MCLT group compared to skin incision group at week 6 between the primary somatosensory cortex hindlimb and mediodorsal nuclei of the thalamus (p=0.016) (Fig. 2B) and the central nucleus of the amygdala (p=.0318) (Fig. 2C). At week 14 we also saw significantly higher connectivity for the female MMT+MCLT animals compared to the female skin incision group for the connection between the left retrosplenial cortex and the left primary somatosensory cortex trunk (p=0.022) (Fig. 2D). Whereas, for males, there was lower functional connectivity in the MMT+MCLT animals compared to skin incision control at week 14 for the connections between the left somatosensory cortex hindlimb and the left retrosplenial cortex (p<0.004) (Fig. 2F), left medial nucleus of the amygdala (p=0.023) (Fig. 2G), and left anterior cingulate cortex (p=0.045) (Fig. 2H). Joint remodeling was confirmed using histological analysis. The top of Fig. 3 shows representative images of the skin incision sham and MMT-MCLT groups, respectively. The representative images illustrate the cartilage loss, increase in subchondral bone, and osteophyte formation in the MMT+MCLT image. The probabilistic heat maps in the bottom of Fig. 3 further illustrate the increases in subchondral bone and decrease in cartilage. Regions with a value closer to 1 indicate a higher probability of tissue – cartilage or subchondral bone in this case – occurring across all animals in the respective groups. Therefore, we can qualitatively observe thinning of the cartilage closer to the medial margin of the tibial plateau, potential thickening of the subchondral bone plate and increases in subchondral bone near the medial margin and anterior collateral ligament insertion site.

DISCUSSION: Characterizing brain changes in OA progression in preclinical models is important for future work to help identify mechanisms through which brain remodeling is occurring in patients. In this study, we show changes in tactile sensitivity, functional connectivity, and joint-level remodeling. Our functional connectivity analyses focused on regions related to 1) sensory input (somatosensory cortex and thalamus), 2) sensory perception (somatosensory cortex and retrosplenial cortex), 3) nociception (somatosensory cortex and amygdala), and 4) the emotional aspect of pain (somatosensory cortex, anterior cingulate cortex, and periaqueductal gray). These results indicate that circuits involved in sensory and nociceptive processing are altered with this model of post-traumatic OA. However, more analysis is needed to determine if any strong correlations exist between functional connectivity, tactile sensitivity, and joint remodeling. Additional work will also investigate motor and cognitive effects of OA in preclinical rodent models using MRI, gait analysis, and cognitive behavior assays. We seek to understand the disconnect between pain processing and pain-related behavior. Additional work is planned to investigate MRI data related to cognition and motor control paired with cognitive behavior and gait analysis.

SIGNIFICANCE/CLINICAL RELEVANCE: This study establishes a foundation for understanding the relationship between joint remodeling, brain remodeling and pain related behavior in an aging rodent model of OA. By understanding this relationship in preclinical models, the effects of OA progression on the brain can be better characterized.

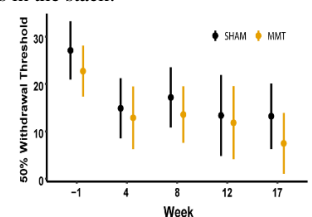


Fig 1. Tactile sensitivity data

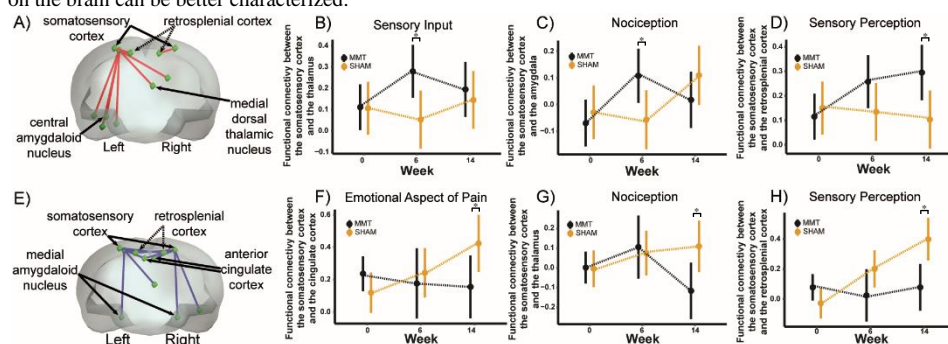


Fig 2. Functional connectivity maps for A) female rats and E) male rats. Red connections illustrate increases and blue indicates decrease. Corresponding functional connectivity plots for sensory input (B), nociception (C and G), sensory perception (D and H), and the emotional aspect of pain (F).

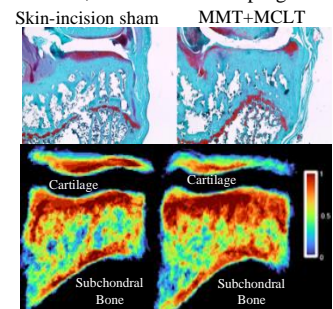


Fig 3.A) Representative images of joint histology. B) Probabilistic heat maps showing location dependent decreases in cartilage and increases in subchondral bone.