**Introduction**

Cancer statistics are equally puzzling and humbling—in 2018, over 1.7 million new cancer cases and 600,000 cancer-related deaths are projected to occur in the United States alone. Though grim, these statistics do not speak to the concurrent 76% drop in combined US cancer deaths since the early 1990s. The benefits of improving cancer treatments and decreasing mortality rates have given rise to a global survivor population of over 32 million. Chemotherapy is an indispensable treatment option for many common cancers and is used in neoadjuvant, adjuvant, and metastatic settings. Unfortunately, chemotherapy-induced peripheral neuropathy (CIPN) affects as many as 50% of patients treated with combination chemotherapies. CIPN is characterized by chronic burning or burning pain and loss of sensation that begins at the extremities and progresses more centrally; many also report mechanical sensitivity and thermal allosthenia/hyperalgesia. The present study in rats uses diffusion weighted imaging with quantitative anatasy to follow changes in gray matter microarchitecture and resting state functional connectivity to follow the functional reorganization of neural circuits involved in pain perception following paclitaxel treatment.

**Materials & Methods**

**Experimental Design**

Male Sprague Dawley rats (n = 16) weighing between 300-325 gm were obtained from Charles River Laboratories (Wilmington, Massachusetts, USA). Studies were performed on a Bruker Biopol 7 T / 35cm URE. These three were acquired 1) High resolution Anatomy scans, 2) Diffusion Tensor Imaging 3) Functional Connectivity scans.

**Diffusion Tensor Imaging**

DTI was acquired with a diffusion-weighted (DW) spin-echo echo-planar imaging (EPI) pulse sequence having the following parameters: TR/TE=1000/15 ms, voxel size=0.312 x 0.312 x 0.12 mm. Data was analyzed using Analysis of Functional Neuroimages (AFNI) (http://afni.nimh.nih.gov/afni/), FMRIB Software library (FSL, v5.0.9, TR/TE=1000/15 ms, voxel size=0.312 x 0.312 x 0.12 mm). Data was analyzed using Analysis of Functional Neuroimages (AFNI) (http://afni.nimh.nih.gov/afni/), FMRIB Software library (FSL, v5.0.9, TR/TE=1000/15 ms, voxel size=0.312 x 0.312 x 0.12 mm). Data was analyzed using Analysis of Functional Neuroimages (AFNI) (http://afni.nimh.nih.gov/afni/), FMRIB Software library (FSL, v5.0.9, TR/TE=1000/15 ms, voxel size=0.312 x 0.312 x 0.12 mm). Data was analyzed using Analysis of Functional Neuroimages (AFNI) (http://afni.nimh.nih.gov/afni/), FMRIB Software library (FSL, v5.0.9, TR/TE=1000/15 ms, voxel size=0.312 x 0.312 x 0.12 mm). Data was analyzed using Analysis of Functional Neuroimages (AFNI) (http://afni.nimh.nih.gov/afni/), FMRIB Software library (FSL, v5.0.9, TR/TE=1000/15 ms, voxel size=0.312 x 0.312 x 0.12 mm). Data was analyzed using Analysis of Functional Neuroimages (AFNI) (http://afni.nimh.nih.gov/afni/), FMRIB Software library (FSL, v5.0.9, TR/TE=1000/15 ms, voxel size=0.312 x 0.312 x 0.12 mm).

**Resting State Functional Connectivity**

Images were acquired using a single-shot EPI sequence with matrix size 256x256x256, TR/TE=1000/15 ms, voxel size=0.312 x 0.312 x 0.12 mm. Data was analyzed using Analysis of Functional Neuroimages (AFNI) (http://afni.nimh.nih.gov/afni/), FMRIB Software library (FSL, v5.0.9, TR/TE=1000/15 ms, voxel size=0.312 x 0.312 x 0.12 mm). Data was analyzed using Analysis of Functional Neuroimages (AFNI) (http://afni.nimh.nih.gov/afni/), FMRIB Software library (FSL, v5.0.9, TR/TE=1000/15 ms, voxel size=0.312 x 0.312 x 0.12 mm). Data was analyzed using Analysis of Functional Neuroimages (AFNI) (http://afni.nimh.nih.gov/afni/), FMRIB Software library (FSL, v5.0.9, TR/TE=1000/15 ms, voxel size=0.312 x 0.312 x 0.12 mm).

**Drug**

Paclitaxel was purchased from Trexel Corporation (Virginia, CA). Paclitaxel was dissolved in a vehicle consisting of ethanol:glycerol:water (90:5:5, v/v/v). Doses were determined by intraperitoneal (i.p.) injection of 2 mg/kg/dose and a cumulative dose of 8 mg/kg i.p. for each rat. The doses were administered to a group of rats. The doses were administered to a group of rats. The doses were administered to a group of rats. The doses were administered to a group of rats. The doses were administered to a group of rats. The doses were administered to a group of rats.

**Cold Plate Assay**

The cold plate assay to test for cold allodynia was taken from Brenner and coworkers and modified for use in rats. Prior to testing, all rats were acclimated to standing on a ¼ inch glass plate table, confined to an area of 38 x 34 cm by a transparent plastic border. After acclimatization, each rat was tested for 5 min. A cold plate with a tip dimension of 2 mm was filled with compressed, crushed dry ice. The center of the plantar surface of the hindpaw was targeted for stimulation through the center of the glass plate. The cold probe was positioned on each hindpaw with an interval of 7 min between each test. Both hindpaws of each rat were stimulated for a total of 15 minutes between trials. The latency to move the hindpaw away from the cold probe was timed in seconds. The maximum time allowed for withdrawal was 90 sec. Withdrawal latencies were averaged for each rat and then corrected for the median raphe and periaqueductal gray (highlighted in blue). The median raphe and periaqueductal gray (highlighted in blue) were identified as areas of significant functional changes following vehicle or paclitaxel treatment to the dorsal medial striatum. The median raphe and periaqueductal gray (highlighted in blue) were identified as areas of significant functional changes following vehicle or paclitaxel treatment to the dorsal medial striatum. The median raphe and periaqueductal gray (highlighted in blue) were identified as areas of significant functional changes following vehicle or paclitaxel treatment to the dorsal medial striatum. The median raphe and periaqueductal gray (highlighted in blue) were identified as areas of significant functional changes following vehicle or paclitaxel treatment to the dorsal medial striatum.

**Putative Pain Neurocircuit**

The significant functional connections following vehicle or paclitaxel treatment to the dorsal medial striatum, paclitaxel treatment shows enhanced positive connectivity in the basal ganglia and limbic ctx and anticorrelation in the cerebellum/brainstem. The 3D organization of these brain areas are shown in the glass brains.

**Summary**

Within eight days of paclitaxel treatment and induction of neuropathic pain, diffusion weighted imaging identified a constellation of brain areas e.g. prefrontal ctx, amygdala, hippocampus, hypothalamus and the stria/accumbens dopaminergic system involved in the emotional and motivational response to chronic pain. This putative reorganization of gray matter microarchitecture forms a continuum of brain areas stretching from the basal medial/lateral forebrain to the midbrain. Resting state FC identified alterations in connectivity in the brainstem, particularly between nodes of the pain neural circuitry. Unlike previous studies in rat neuropathic pain models, paclitaxel treatment affects reorganization of pain neural circuitry, particularly the PAG. These data using different imaging modalities fit the theory that chronic pain reorganization is a continuum and that influence activity in the PAG and brainstem to modulate pain perception.

**Caveat**

There are limitations that should be noted. 1) These data were collected from a single time immediately after one course of paclitaxel. A comparison to data collected months later with more profound effects will show how the patterns of reorganization. 2) Immunohistochemical data on neuroinflammation or changes in the extracellular matrix would help to understand the mechanism behind the changes in connectivity. 3) The role of growth factors collected under low dose inflammation arrest motor axon and neuronal dysfunction (27). Nonetheless, these changes comparing the anesthetized and conscious state shows that diffuse cingulate paclitaxel treatment to the dorsal medial striatum...