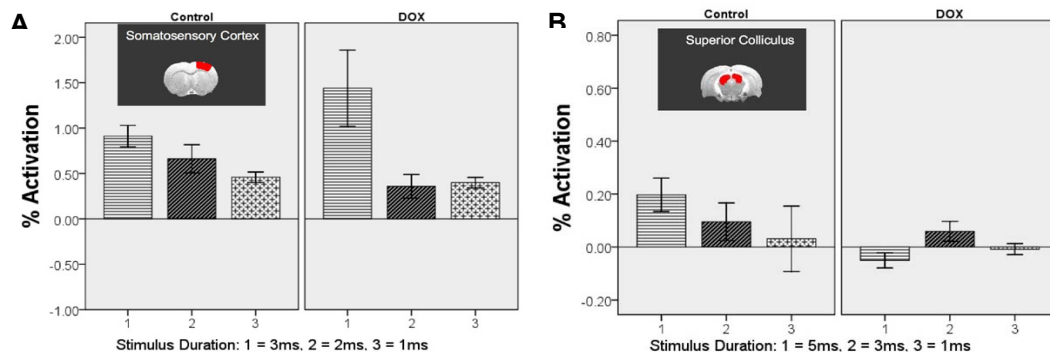


## Effects of Doxorubicin on Brain Activity and Functional Connectivity in Rats

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**INTRODUCTION.** Adjuvant chemotherapy is commonly included in the therapy of patients being treated for breast cancer in order to decrease the incidence of recurrence. Several different treatment paradigms are used, but most include an anthracycline such as doxorubicin. There is a growing literature demonstrating that adjuvant chemotherapy administration for breast cancer and other malignancies induces cognitive impairment in a significant proportion of patients. This has often been referred to as chemobrain. Little is known regarding its cause at a mechanistic level, or even its duration and/or permanence. Rodent models for the study of the toxicity of doxorubicin and other chemotherapeutic agents have been developed. We are investigating "chemobrain" at a mechanistic level by determining the effects of doxorubicin on brain function using fMRI and resting state functional



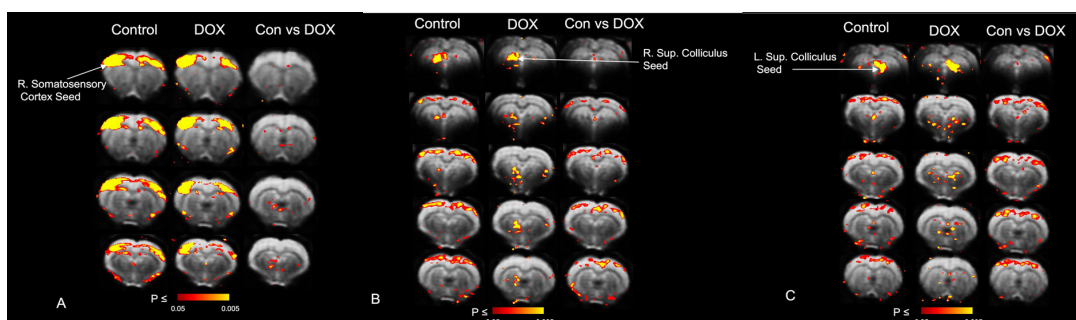
**Figure 1.** Effects of weekly doses of doxorubicin (DOX 1mg/kg) on brain activation by stimulation of the forepaw in FSSC (A) and by visual stimulation in the SC (B)

electrical stimulation of the forepaw (SS) on somatosensory activation and flashing light stimulation (VIS) of the eye on visual system activation were determined using BOLD fMRI. Electrical stimulation of the forepaw produced strong intensity-related activation in the ipsilateral forelimb somatosensory cortex (FSSC) and in a small region of the striatum. Visual stimulation produced bilateral activation of the superior colliculus (SC), lateral geniculate (LG) and a small region of activation in primary visual cortex (PVC). When mean activation intensity is computed in each anatomical region (Fig. 1), it can be seen that both SS (A) and VIS (B) produce activation that increases parametrically with stimulus intensity in control rats. This is altered significantly ( $p \leq 0.05$ ) by repeated weekly treatments with DOX when measured after 4 to 7 weeks of treatment. DOX increased the activation by the

connectivity MRI (fcMRI) in female rats by examining the effects of weekly treatment with doxorubicin on brain activation induced by somatosensory and visual stimulation.

**METHODS AND RESULTS .** Basically, nine female control rats and 5 rats treated once a week (4 to 7 weeks) with 1 mg/kg iv of DOX were studied. One week later they were prepared for imaging. Scanning was performed using a Bruker, 9.4 T scanner. Rats were anesthetized with dexmedetomidine and artificially respired after paralysis with pancuronium. The effects of three intensity levels of

highest intensity SS, but decreased activation by the lower two intensities. DOX treatment decreased activation by VIS at all three stimuli intensities. A similar pattern was seen in the LG.



**Figure 2.** Effects of weekly DOX treatment on fcMRI connectivity in the brains of female rats. Three functional seed ROIs were defined based on the group SS and VIS fMRI data. The ROIs were from the (A) right forelimb somatosensory cortex, (B) right and (C) left superior colliculus of interest to isolate regions that are functionally correlated. rats. A two-sample t-test was done to then compare the groups. All group maps are thresholded at  $p < 0.05$ .

Functional connectivity (fcMRI) studies have provided insight into the intrinsic functional architecture of the brain, variability in behavior, and potential physiological correlates of neurological and psychiatric diseases and could give further insight into mechanisms underlying the effects of chemotherapeutic drugs on cognition. We examined resting state fcMRI in the same groups of rats by performing a 6 min single-shot EPI sequence

(slice thickness 1 mm, TR = 2000 ms) resting state scan prior to any sensory stimulation. Images were motion corrected and temporally band pass filtered ( $0.01 < f < 0.08$ ). The global signal from the brain was regressed from each voxel as spurious noise. Three seed ROIs (right FSSC and the right and left SC) were defined based on the group SS and VIS fMRI data. Mean time-course signal from each ROI was then used as a regressor of interest to isolate regions that are functionally correlated. Correlation coefficients between the seed and all other voxels were then transformed to normally distributed Fisher Z values for statistical comparisons. One-sample t-tests were performed and results are shown in fig. 2. It can be seen in Figure 2A that there is significant correlation between resting state MR signal patterns in the FSSC seed area and both the surrounding cortex and the contralateral FSSC. There were no regions that were consistently significantly different between the two groups. However, in ROIs associated with the visual system, significant decreases in resting state functional connectivity were observed in the DOX group. The right SC is the seed region in 2B and the left in 2C. There is functional connectivity between the seed regions and bilateral PVC in the control group. This is significantly diminished in the DOX group. This is consistent with our finding in of diminished activation by visual stimulation, in the DOX group. This supports our hypothesis that treatment with DOX will cause changes in neuronal functional activity that can be detected and quantified using fMRI and resting state fcMRI methods.

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