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Award Number: W81XWH-09-1-0597

TITLE:

Effects of Breast Cancer Chemotherapy Agents on Brain Activity
in Rats: Functional Imaging Studies

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REPORT DATE: April 2011

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE (DD-MM-YYYY) 29/04/2011		2. REPORT TYPE Final		3. DATES COVERED (From - To) 30 Sep 2009 - 29 Mar 2011	
4. TITLE AND SUBTITLE Effects of Breast Cancer Chemotherapy Agents on Brain Activity in Rats: Functional Imaging Studies				5a. CONTRACT NUMBER W81XWH-09-1-0597	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Alan S. Bloom, Ph.D. abloom@mcw.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Medical College of Wisconsin Milwaukee, WI 53226-3548				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Material Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT There is an increasing awareness that adjuvant chemotherapy administration for breast cancer and other malignancies can cause cognitive impairment or "chemobrain" in a significant proportion of patients. Little is known regarding its cause or even its duration and/or permanence. Rodent models are available for studying toxicity from doxorubicin and other chemotherapeutic agents and behavioral impairment has been demonstrated using them. The overall goal of this project is to investigate this impairment at a mechanistic level by determining the effects of doxorubicin, one of the agents commonly used for the adjuvant chemotherapy of breast cancer, on brain function using functional magnetic resonance imaging (fMRI) and behavioral techniques in a rat model. Female rats are treated with doxorubicin weekly, for six weeks. One week later, they are behaviorally tested and imaged using fMRI. Measures will also be made in rats, one and three months after doxorubicin treatment. To date, we have observed that treatment with doxorubicin alters brain activation in response to both visual and somatosensory stimulation with the effects greatest in the visual system. In addition, doxorubicin significantly decreases functional connectivity in the rat visual system. This supports our hypothesis that treatment with doxorubicin will cause changes in neuronal functional activity that can be detected and quantified using fMRI methods. Furthermore, it is consistent with impaired visual cognitive processing reported after chemotherapy.					
15. SUBJECT TERMS Cancer chemotherapy, animal model, cognitive impairment, fMRI					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 11	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

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INTRODUCTION:

We have carried out studies using high-field (9.4T) fMRI to mechanistically examine the effects of adjuvant chemotherapy commonly used for breast cancer treatment on brain function in a rat model. 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 [1-3]. This has often been referred to as chemobrain or chemo fog. At present, little is known regarding its cause at a mechanistic level, or even its duration and/or permanence. Similarly, there are no generally accepted treatments to either prevent or ameliorate the cognitive effects of cancer chemotherapy. Rodent models for the study of the toxicity of doxorubicin and other chemotherapeutic agents have been developed and behavioral impairment has been demonstrated using them [4, 5]. It is the overall goal of this project to investigate this at a mechanistic level by determining the effects of doxorubicin, one of the agents commonly used for the adjuvant chemotherapy of breast cancer, on brain function using fMRI and behavioral techniques in a rat model. It is hoped that this model will enable us to ultimately determine the duration of and mechanism for the effects of doxorubicin on brain function and to ultimately evaluate the efficacy of possible neuroprotective or other agents in preventing or treating chemobrain.

BODY:

Effects of Doxorubicin (DOX) on brain activation by somatosensory and visual stimulation. We have carried out studies in female rats examining the effects of weekly treatment with doxorubicin on brain activation induced by somatosensory and visual stimulation. The methods are detailed below. Female control rats (225 g) and rats treated once a week (4 to 7 weeks) with 2.5 mg/kg iv of DOX were studied. In One week later they were prepared for imaging. Scanning was performed using our 30 cm, 9.4 T scanner. Rats were anesthetized with dexmedetomidine and artificially respiration after paralysis with pancuronium. It has been demonstrated at this institution that this paradigm allows for near normal heart rate, BP and blood gases and preserves brain fMRI response to sensory stimulation and functional connectivity[6, 7].

The adjacent figures shows the effects of three intensity levels of electrical stimulation of the forepaw (SS) on activation in the somatosensory cortex and flashing light stimulation (VIS) of the eye on activation in the superior colliculus. Figure 1 shows the regions that were significantly activated (corrected $p < 0.05$) by the forepaw (a) and visual (b) stimulation along with representative activation patterns from 9 adjacent voxels in the region. It can be seen that electrical stimulation of the forepaw produces strong intensity-related activation in the ipsilateral forelimb somatosensory cortex and in a small region of the striatum. Visual stimulation produced bilateral activation of the superior colliculus, lateral geniculate and a small region of activation in primary visual cortex.

When mean activation intensity is computed in each anatomical region (Fig 2.), 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

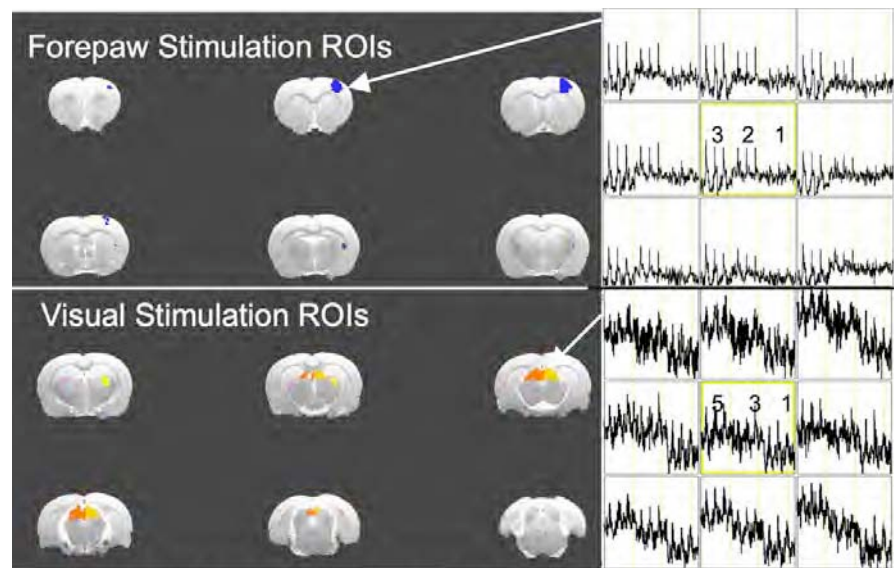


Figure 1. Significantly ($p \leq 0.05$) activated regions from control rats ($n=9$) from electrical stimulation of the forepaw (current – 1.5 mA, pulse duration – 1, 2 and 3 ms, repetition rate – 3 Hz) or visual stimulation (rate – 5, 3 or 1 Hz). Time courses from typical rats are from 9 adjacent voxels in the ROIs as indicated by the arrows and are concatenated across the three stimulus intensities. A stimulation paradigm of 20 sec on and 40 sec off repeated 3 times is used for each intensity.

repeated weekly treatments with DOX. DOX increased the activation by the highest intensity SS by 62%, but decreased activation by the lower two intensities by 49% and 19% respectively.

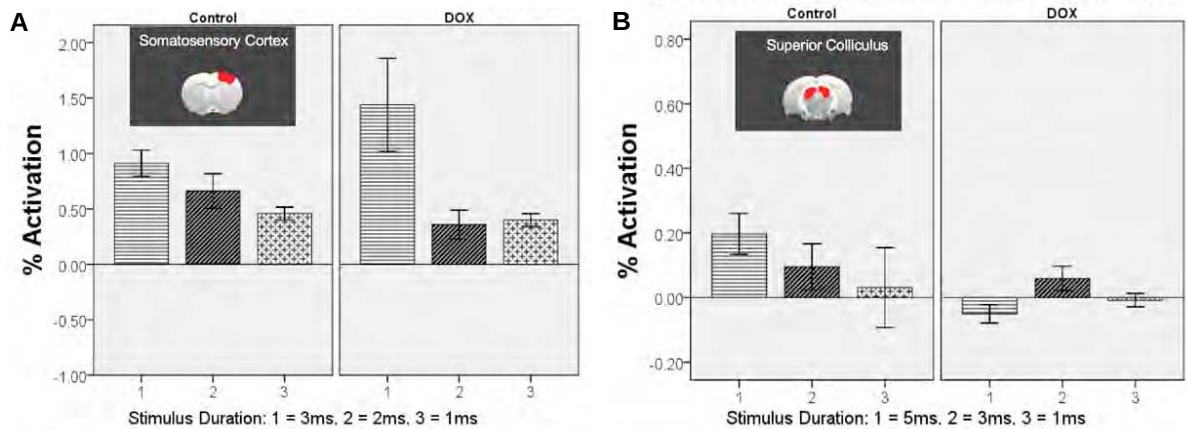


Figure 2. Effects of weekly doses of 2.5 mg/kg doxorubicin (DOX) on brain activation by electrical stimulation of the forepaw in somatosensory cortex (A) and by visual stimulation in the superior colliculus (B). $N=9$ for the control group and 5 for DOX.

DOX treatment strongly decreased activation by VIS at all three stimuli intensities. A similar pattern was seen in the lateral geniculate.

These results demonstrate the feasibility of using brain activation by parametric sensory stimulation as measured by BOLD fMRI as being sensitive to the effects of DOX administration and as an appropriate tool to determine the duration of effects and the ability of antioxidants and other drugs to modify DOX-induced effects.

Effects of Doxorubicin on Global and Regional CBV and CBF

Whole brain and functional region of interest (ROI) measurements of cerebral blood flow (CBF) and relative cerebral blood volume (rCBV) were made in the control and DOX treated rats reported on in figs. 2 and 3. after the completion of the resting state and sensory activation scans described above were completed. Briefly, for perfusion scanning, a set of pre-contrast images is acquired using a fast-spoiled gradient recalled echo (SPGR) sequence. Next, The perfusion scan is carried out with Multihance (0.1 mmole/kg) injected during the acquisition, followed by a post contrast scan. Image processing is described below. There was a slight but not significant 12% decrease in whole brain CBF which may be driven by significant ($p \leq 0.05$) decreases in both the right and left lateral geniculate functional ROIs (25% and 29%, respectively). There were smaller but not statistically significant decreases in the other visual system ROIs (superior colliculus and primary visual cortex). A similar pattern was also observed for rCBV with a significant 28% decrease in rCBV in the left lateral geniculate and a smaller (17%) non-significant decrease in the right lateral geniculate. These differences are interesting in view of the greater impairment of VIS stimulation-induced activation compared to SS-induced activation after DOX treatment. Since this is a resting state measurement, there are implications for the interpretation of BOLD fMRI measurement in these animals. If this holds true in future studies, we will need to account for the changes in perfusion in our calculation of task activation.

Functional Connectivity (fcMRI) Studies. Functional connectivity (fC) 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 this group of rats by performing a 6 min (A single-shot EPI sequence (FOV=3.5 cm, slice thickness 1 mm, image matrix = 96x96, zero filled to 128x128, TR=2000 ms, TE=19 ms, TR = 2000 ms) resting state scan prior to any sensory stimulation. Three functional seed ROIs were defined based on the group SS and VIS fMRI data. The functional ROIs were from within the right forelimb somatosensory and the right and the left superior colliculus cortex. Mean time-course signal from each ROI was then used as a regressor of interest to isolate regions that are functionally correlated. One-sample t-tests were performed and results are shown in figure 3. A two sample t-test was done to then compare the groups. All group maps are thresholded at $p < 0.05$. It can be seen in Figure 3A that there is significant correlation between resting state MR signal

patterns in the somatosensory cortex seed area and both the surrounding cortex and somatosensory cortex in the contralateral hemisphere.

There were few 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 superior colliculus (involved in visual system reflexes, etc.) is the seed region in 3B and the left in 3C. There was widespread functional connectivity between the seed regions and bilateral visual cortex in the control group. There is significantly diminished connectivity between seed ROI and cortex in the DOX group when compared to the control group. This is consistent with our finding above 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 methods. Furthermore, it is consistent with impaired visual cognitive processing reported after chemotherapy [8].

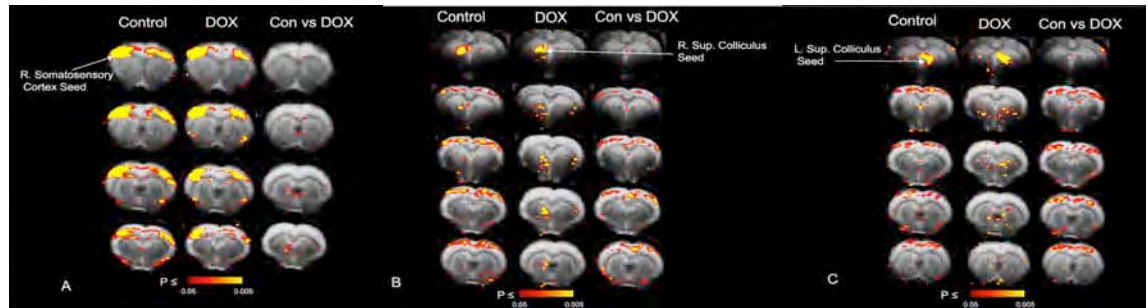


Figure 3. Effects of weekly DOX treatment on fcMRI connectivity in the brains of female rats. Three functional seed ROIs were defined based on the group SS and VIS fMRI data. The functional ROIs were from within the (A) right forelimb somatosensory cortex, (B) right and (C) left superior colliculus of interest to isolate regions that are functionally correlated. Correlation coefficients between the seed and all other voxels were then transformed to normally distributed

Effects of DOX on rotarod and novel object test behavior.

The effects of the above DOX treatment on performance in the rotarod test and novel object tests were also examined. the rotarod test of motor skill learning is sensitive to motor skill learning and not just general levels of fitness. Since skill learning is typically associated with the cerebellum and motor pathways, and human studies of PET imaging in chemobrain have reported changes in blood flow in these regions [9], this task may be sensitive to the effects of chemotherapy agents on brain regions involved in locomotor activity and motor skill learning. Animals are placed on the rotarod and receive 10 consecutive training sessions as the rod rotates at 10 rpm. The novel object recognition test measures recognition memory through novel object orientation in rats [10]. According to the paradigm, the test is conducted in an open field containing objects constructed with Duplo Lego blocks placed in 4 distinct locations spaced evenly in a circular field. A habituation, sample trial, and choice trial session occur. During habituation, animals are allowed to explore 2 identical cubes for 15 minutes. During the sample trial, rats are allowed to explore 2 new objects for 5 minutes. Following a delay, animals perform the choice trial. During the choice trial, animals are allowed to explore a novel object and a familiar (previously explored) object for 5 minutes. The dependent measure is amount of time exploring. Object location remains consistent during learning sessions. Behavioral testing is carried out one day prior to scanning. Control rats remained on the rotarod for 124 ± 10 seconds. This was reduced by 31.5% to 85 ± 11 sec in the DOX treated group. In the novel object test, control rats spent 57% more time exploring the novel object compared to the familiar object. This was not seen in the DOX treated group. In addition, the control group traveled an increased distance during the sessions. (They averaged 2325 ± 242 cm compared to 1748 ± 95 cm for the DOX group.) These suggests that the proposed measures are sensitive to the effects of DOX. However we must take differences in general motor activity into account in interpretation of the results.

KEY RESEARCH ACCOMPLISHMENTS:

We have demonstrated in a rat model of DOX toxicity:

- DOX treatment alters brain activation by sensory stimulation particularly in regions associated with visual systems.
- Resting state functional connectivity MRI is quantifiable in the dexmedetomidine anesthetized rat and that functional connectivity is decreased by DOX treatment, particularly in the visual system.
- We consistently see functional impairment in the visual system compared to the somatosensory system in the rat. Although this is not one of the most commonly reported symptoms of human “chemobrain”, it is consistent with impaired visual cognitive processing reported after chemotherapy [8]. This may give us a tool that is needed to compare other indicators of DOX toxicity, such as oxidative stress in the two systems

REPORTABLE OUTCOMES:

During this single year project we have submitted 3 abstracts, made 2 presentations at national meetings and will be making a third this summer. We have also used the data gathered from this Concept award as preliminary pilot data for the submission of program project grant to the NCI. the title of the proposal is “New Paradigms for Breast Cancer Therapy Optimized with Advanced Imaging”. The project associated with this award is “Effects of Doxorubicin on Brain Activity in Rats: Functional Imaging Studies” It received the best score of the study section that reviewed it, and we are awaiting a funding decision.

I. Abstracts and meeting presentations.

Bloom, A.S., LaViolette, P.S., Chitambar, C.R., Collier, W., Durgerian, S.J., Kalyanaraman, B., McAllister, D.M., Williams, C.L., Schmainda, K.M. Effects of Doxorubicin on Brain Activity and Functional Connectivity in Rats.

2010 Meeting of the International Society for Magnetic Resonance Medicine, Stockholm, Sweden

A.T. Barr, C.J. Ford¹, P.S. LaViolette, C.R. Chitambar, W. Collier, S.J. Durgerian, L. Piacentine, K.L. Douville S.A. Claesges, B. Kalyanaraman, D.M. McAllister, C.L. Williams, K.M. Schmainda and A.S. Bloom, Breast Cancer Adjuvant Chemotherapy Induced Changes in Functional Connectivity in Female Breast Cancer Patients and a Rodent Model of Chemotherapy,.
Proceedings of the Second Biennial International conference on Resting-State Functional Brain Connectivity, Milwaukee, Wisconsin pp. O18, 2010.

Alan Bloom, William Collier, Sally Durgerian, Peter LaViolette, Balaraman Kalyanaraman, Carol Williams, Kathleen Schmainda Effects of Doxorubicin on Brain Activity and Resting-State Functional Connectivity in Rats: fMRI Studies
2011 Era of Hope Conference (submitted)

II. Grants applied for and pending:

NIH/NCI - Program Project Grant P01CA151134-01 Period 7/01/2011 – 6/30/2016

New Paradigms for Breast Cancer Therapy Optimized with Advanced Imaging
Principal Director – Kathleen Schmainda, Ph.D. Role – Principal Investigator for Project 3 (30% effort)

Has five projects and four cores. Bloom – Bloom is Project Leader for “Effects of Doxorubicin on Brain Activity in Rats: Functional Imaging Studies”
TDC - \$9,714,674.

III. List of personnel:

Alan S. Bloom, Ph.D.

Kathleen, Schmainda, Ph.D.

William Collier

CONCLUSION:

The research conducted under this Concept Award demonstrates the feasibility of using a rat model to study the effects of cancer chemotherapy on brain function and behavior. To date we have found that treatment with the anthracycline cancer chemotherapy agent, doxorubicin alters brain activation by sensory stimulation particularly in regions associated with visual systems. We have also demonstrated that resting state functional connectivity MRI is quantifiable in the dexmedetomidine anesthetized rat and that functional connectivity is decreased by DOX treatment, particularly in the visual system. We consistently see functional impairment in the visual system compared to the somatosensory system in the rat. Although impairment in the visual system is not one of the most commonly reported symptoms of human “chemobrain”, it is consistent with impaired visual cognitive processing reported after chemotherapy. These results demonstrate the feasibility of using brain activation by parametric sensory stimulation and resting-state functional connectivity as measured by BOLD fMRI as being sensitive to the effects of doxorubicin administration and hopefully that of other cancer chemotherapy agents as an appropriate tool to determine drug mechanisms and the ability of antioxidants and other drugs to modify chemotherapy-induced cognitive effects.

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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

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

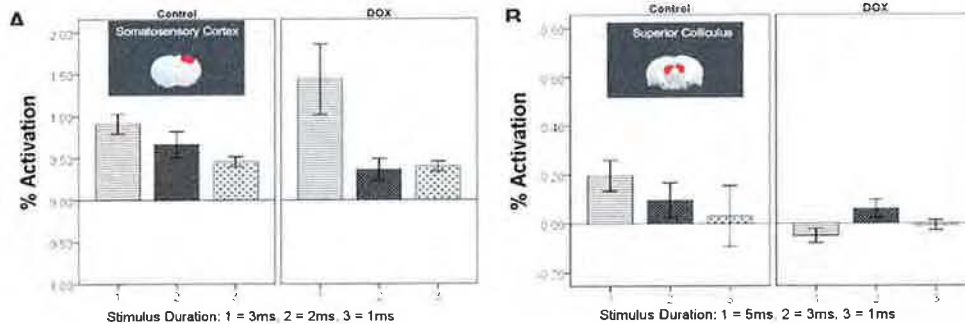


Figure 1. Effects of weekly doses of doxorubicin (DOX 1mg/kg) on brain activation by stimulation of the forepaw in FSSC (A) and by visual stimulation in the SC (B)

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 ($p < 0.05$) by repeated weekly treatments with DOX when measured after 4 to 7 weeks of treatment. DOX increased the activation by the

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.

ACKNOWLEDGEMENTS Funding: MCW Cancer Center

Breast Cancer Adjuvant Chemotherapy Induced Changes in Functional Connectivity in Female Breast Cancer Patients and a Rodent Model of Chemotherapy

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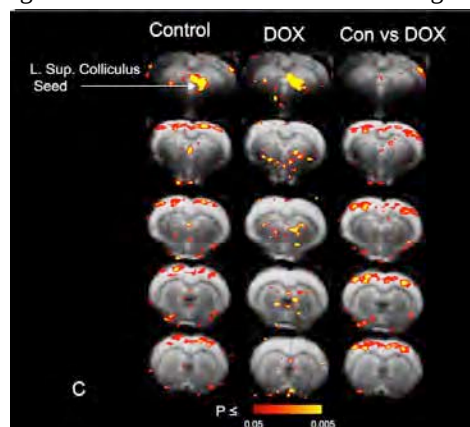
Introduction: Breast cancer patients receiving adjuvant chemotherapy often report cognitive deficits including memory loss, decreased concentration as well as difficulties multi-tasking. The aforementioned symptoms are often referred to as “chemobrain” (CB) or “chemofog”. Although the phenomena is now well recognized, its causality is still not well understood. In order to gain mechanistic insight into CB, we have initiated prospective functional imaging studies of it in both breast cancer patients and a rat model of breast cancer chemotherapy. The studies reported here include resting-state analysis of breast cancer patients both before and after chemotherapy as well as analogous studies in female rats.

Methods and Results

Humans: Female breast cancer patients (n=4) receiving doxorubicin (DOX) based adjuvant chemotherapy (mean age: 46 years; range: 35-62 years) were scanned on a GEHC 3.0T Excite MR scanner prior to receiving chemotherapy and after 4 sessions of chemotherapy, given every 2-3 weeks. Age matched controls (n=4) were scanned using the same schedule. Images were acquired sagittally using a Gradient EPI (TR = 2s). Resting-state images were acquired at the beginning of the scan session during which subjects were instructed to remain awake with eyes open during the scan. The resting state data was motion corrected, smoothed and filtered using a band-pass filter ($12 < f < 111$). Regressors for white matter and ventricles were applied to reduce physiological noise. All subject's images were converted into template space. A seed region was placed in the posterior cingulate cortex (PCC) an area known to be involved in episodic memory. Correlations were found between the seed region and the entire brain on a voxel by voxel-wise basis. Group analysis was performed on the Fisher Z-transform of the correlation coefficients.

In the control group, a significant ($p \leq 0.005$) resting state correlation between the PCC and the medial prefrontal cortex (MPC), part of the dorsal attention system, was seen bilaterally. This was significantly decreased in the pre-chemotherapy scan of the breast cancer group and was further decreased after 4 sessions of chemotherapy. The medial temporal cortex, an area also significantly correlated with the PCC in the control group, was not significantly correlated with the PCC in breast cancer patients neither prior to nor following chemotherapy. Other significant differences were not observed in this small group of patients..

Rats: Female SD rats (n=5) received weekly 1 mg/kg iv injections of DOX for four to seven weeks and control rats (n=9) received weekly injections of saline vehicle. Rats were imaged using a 9.4T Bruker MR scanner (axial EPI scans, TR/TE=2s/19ms). During surgery rats were anesthetized with isoflurane. Following surgery anesthesia was changed to dexmedetomidine and rats, artificially respirated following paralysis with pancuronium. Prior to sensory activation studies, resting state data was acquired for 6 min. The images were aligned to a common dataset and corrected for motion, temporally band-pass filtered and nuisance regressors were removed to decrease signal noise. Fisher Z-transform was applied to the correlation coefficients for the group analysis.



Effect of weekly DOX treatment on fcMRI to l. Sup. colliculus of female rats.. A two-sample t-test was done to then compare the groups. All group maps are thresholded at $p < 0.05$.

Functional connectivity analysis on the resting-state data with seeds in the right and left superior colliculus, part of the visual system and in the right somatosensory cortex were carried out. ROI's were chosen based on somatosensory and visual activation studies acquired during the same scan session. The functional connectivity analysis showed significant ($p \leq 0.05$) correlation between the superior colliculus and the visual cortex and other components of the visual system which was significantly decreased in the DOX treated group (FIG). These findings were consistent with significant decreases in BOLD activation after DOX in the superior colliculus.

Discussion: The functional connectivity analysis of the human patients receiving chemotherapy showed decreased resting state connectivity in areas known to be associated with attention and memory systems. These changes are consistent with deficits reported by patients. DOX-induced decreases in functional connectivity were also observed in the rat model. However, breast cancer patients prior to receiving chemotherapy, already display changes in connectivity in comparison to controls. This suggests that cognitive impairment associated with breast cancer treatment may be multi-factorial in cause. Longer term studies with larger group sizes may help elucidate the changes that are occurring in connectivity and behavior and its cause.