Published in final edited form as:

Brain Behav Immun. 2020 October ; 89: 451–464. doi:10.1016/j.bbi.2020.07.032.

Social Enrichment Attenuates Chemotherapy Induced Pro-inflammatory Cytokine Production and Affective Behavior via Oxytocin Signaling

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Abstract

Breast cancer survivors receiving chemotherapy often report increased anxiety and depression. However, the mechanism underlying chemotherapy-induced changes in affect remains unknown. We hypothesized that chemotherapy increases cytokine production, in turn altering exploratory and depressive-like behavior. To test this hypothesis, female Balb/C mice received two injections, separated by two weeks, of vehicle (0.9% saline) or a chemotherapeutic cocktail [9 mg/kg doxorubicin (A) and 90 mg/kg cyclophosphamide (C)]. Peripheral and central cytokine concentrations were increased one and seven days, respectively, after AC. Because of the beneficial effects of social enrichment on several diseases with inflammatory components, we examined whether social enrichment could attenuate the increase in peripheral and central cytokine production following chemotherapy administration. Socially isolated mice receiving AC therapy demonstrated increased depressive-like and exploratory behaviors with a concurrent increase in hippocampal IL-6. Whereas, group housing attenuated AC-induced IL-6 and depressive-like behavior. Next, we sought to determine whether central oxytocin may contribute to the protective effects of social housing after AC administration. Intracerebroventricular administration of oxytocin to socially isolated mice recapitulated the protective effects of social enrichment; specifically, oxytocin ameliorated the AC-induced effects on IL-6 and depressive-like behavior. Furthermore, administration of an oxytocin antagonist to group housed mice

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AUTHOR CONTRIBUTIONS

WHWII designed, completed, analyzed all experiments and wrote the manuscript; OHMF, JLP, NZ conducted experiments and provided editorial comments on the paper; ACD designed experiments and wrote and edited the manuscript.

CONFLICTS OF INTEREST

The authors do not have any conflicts of interest to report.

DATA AVAILABILITY

The data that support the findings of this study are available (in raw form) from the corresponding author upon reasonable request.

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recapitulated the responses of socially isolated mice; specifically, AC increased depressive-like behavior and central IL-6. These data suggest a possible neuroprotective role for oxytocin following chemotherapy, via modulation of IL-6. This study adds to the growing literature detailing the negative behavioral effects of chemotherapy and provides further evidence that social enrichment may be beneficial to health.

Keywords

Chemotherapy; Inflammation; Affective Behavior; Social Enrichment; Oxytocin

1. INTRODUCTION

According to the National Cancer Institute SEER database, diagnosis of new breast cancer cases have been rising approximately 0.3% each year over the last decade, whereas death rates have been falling approximately 1.5% each year over the same period, likely reflecting improved screening and treatments (Siegel et al., 2020; Webb et al., 2004). Chemotherapy is a mainstay in cancer treatment; the CDC estimates that approximately 650,000 cancer patients will receive chemotherapy in an outpatient setting each year. Although chemotherapy has demonstrated superior anti-tumor efficacy and greatly increased survival rates, it is not without negative side effects. Patients receiving chemotherapy commonly experience nausea, vomiting, diarrhea, stomatitis, alopecia, neutropenia, thrombocytopenia, and myalgias (Partridge et al., 2001). Furthermore, the negative side effects of chemotherapeutics are not limited to somatic symptoms; patients receiving chemotherapy commonly demonstrate an increase in fatigue, depression, and anxiety symptoms, as well as impaired cognitive function and disrupted sleep (Chen et al., 2008; Hipkins et al., 2004; Liu et al., 2009, 2012; Pandey et al., 2006; Wefel and Schagen, 2012). In contrast to animal models, patients receiving chemotherapy typically are aware of their cancer diagnosis, which may be associated with fears of mortality, and thus highly likely to contribute to depression and anxiety symptoms. Notably, the amount of social support prior to cancer treatment predicts the levels of cognitive dysfunction and depressive symptoms in cancer survivors (i.e. low social support increases cognitive dysfunction and depressive symptoms) (Hughes et al., 2014; Reid-Arndt et al., 2010). Affective and cognitive changes are of major concern because alterations in depression, cognitive function, and sleep have been associated with decreased quality of life, decreased survival rates, and increased cancer progression (Bower, 2008; Chen et al., 2008; Li et al., 2008; Pinquart and Duberstein, 2010; Reich et al., 2008; Sandadi et al., 2011; Satin et al., 2009).

Whereas several studies have investigated chemotherapy induced neuropsychological alterations in both the clinical and basic science settings, the precise mechanism of chemotherapy-induced neuropsychological effects remains unknown. Common hypotheses to explain these deficits include increased pro-inflammatory cytokine signaling, oxidative stress, decreased blood flow, decreased neurotrophic factors, decreased neurogenesis, and variability in genes (e.g., apolipoprotein E) that regulate neuronal signaling and glucose metabolism (Ahles and Saykin, 2007; Bower et al., 2011; Egeland et al., 2017; Liu et al., 2012; Palesh et al., 2012; Seigers and Fardell, 2011).

The current study examines the effects of chemotherapy treatment on cytokine production and behavior in tumor free Balb/C mice. We used two of the most commonly used chemotherapeutic drugs for the treatment of breast cancer; the DNA intercalator doxorubicin (Adriamycin; A) and the alkylating agent cyclophosphamide (C) (Cleator et al., 2006). The treatment regimen mirrored two cycles of dose-dense AC therapy (Citron et al., 2003). First, we sought to assess when peripheral and central cytokine production emerge following AC administration. Next, we utilized this information in the second study and chose the timepoint with greatest amount of pro-inflammatory cytokine signaling to determine the effects of social enrichment on peripheral and central cytokine production and depressive-like behavior following AC treatment. Due to the demonstrated beneficial effects of social enrichment on many disease outcomes (Craft et al., 2005; Karelina and Devries, 2011; Maunsell et al., 1995; Norman et al., 2010; Norman et al., 2010b; Waxler-Morrison et al., 1991), we hypothesized that group housing would attenuate the negative side effects of chemotherapy.

2. METHODS

2.1 Animals

All experiments were approved and conducted in accordance with guidelines set by the West Virginia University Institutional Animal Care and Use Committee. Adult (>8 weeks) female Balb/C mice were acquired from Charles River Laboratories and placed in a (14:10) light/dark cycle. Mice were group housed (n=5 per cage) and allowed *ad libitum* access to food (Envigo Teklad #2018) and water purified by reverse osmosis. Prior to any experimental manipulation, mice acclimated to the facility for one week. They were ovariectomized to eliminate the confound of acute ovarian failure in response to chemotherapy administration (Meirow et al., 2004, 2005). In preparation for surgery, mice were anesthetized using 2% isoflurane and placed on their back. A midline incision (1cm) was made allowing for the movement and visualization of each ovarian fat pad. A small (0.5cm) incision was made directly over the ovarian fat pad and the fat pad was withdrawn to allow for visualization of the ovary and uterine horn. Each ovary was removed from the uterine horn via cauterization and uterine horn was returned into the abdominal cavity; the contralateral ovary was removed as described. Following removal of both ovaries, the skin incision was closed using tissue glue. Of note, mice continued to be group housed (n=5) during the one week recovery following ovariectomy procedure.

Experiment 1-Time Course of Cytokine Production Following AC Therapy—

Following one week recovery from ovariectomy, the mice were individually housed, and then one week later received their first injection of vehicle (0.9% saline) or chemotherapeutic cocktail (9 mg/kg doxorubicin and 90 mg/kg cyclophosphamide; IV tail vein). The chosen dose of chemotherapeutic cocktail was based on our previous studies and represents 50% of the weekly human equivalent dose recommended by the National Comprehensive Cancer Network treatment guidelines for the treatment of invasive HER2-breast cancer; human equivalent dose was calculated using body surface area (Borniger et al., 2017; Reagan-Shaw et al., 2008). Fourteen days after the first injection, mice received a second injection of the vehicle or chemotherapeutic cocktail. A blood sample was collected

from the submandibular vein, and then the mice were euthanized for tissue collection one day (n=15), three days (n=15), five days (n=15), or seven days (n=15) following the second injection of vehicle or chemotherapeutic cocktail. Spleens and adrenal glands were removed and weighed. Brains were extracted and placed in a 1.5ml tube containing RNAlater solution and stored at -80°C overnight. (Invitrogen, Waltham, MA). The next day the hippocampus was extracted and placed in a 1.5ml tube for subsequent cytokine analysis via the Meso Scale Discover V-Plex Pro-inflammatory Mouse Panel. Note, the hippocampus was selected as the brain region of interest due to (1) the demonstrated alterations in hippocampal structure and volume in breast cancer survivors that have received chemotherapy (Apple et al., 2017; Bergouignan et al., 2011; Kesler et al., 2013), (2) the demonstrated inflammatory effects of chemotherapeutics within the hippocampus (Borniger et al., 2017; Groves et al., 2017), (3) the high density of microglia and vasculature within the hippocampus (Quintana et al., 2019; Tan et al., 2020), and (4) the described role of the hippocampus in the etiology of depression (Campbell and MacQueen, 2004; Liu et al., 2017)

Experiment 2- Effects of Social Enrichment on Chemotherapy-Induced Cytokine Production and Behavioral Deficits

—Following one week recovery from ovariectomy, mice were randomly assigned to treatment groups, placed into their assigned housing (single or triad housing) and then one week later received their first injection of vehicle (0.9% saline) or chemotherapeutic cocktail (9 mg/kg doxorubicin and 90 mg/kg cyclophosphamide). Note, mice in triad housing were grouped with same-sex, age-matched, ovariectomized familiar Balb/C mice. Fourteen days later mice received their second injection of vehicle or chemotherapeutic cocktail. Seven days following the second injection a subset of mice underwent behavioral testing (open field followed by forced swim test) during the dark phase of their light cycle. Approximately 2 h after behavioral testing, a submandibular blood sample was obtained, and then the mice were euthanized and spleens and adrenal glands removed and weighed. The brains were extracted and split into the left and right hemispheres; one hemisphere was placed in RNA later (Invitrogen, Waltham, MA) and processed for qRT-PCR, while the other hemisphere was post-fixed in 4% paraformaldehyde overnight, and then transferred to a 30% sucrose solution. After cryoprotection, brains were frozen at -80°C until sectioning.

Experiment 3- OT Administration and Experiment 4- OTA Administration

—One week following the ovariectomy procedure, the mice were randomly assigned to experimental groups and an Alzet osmotic mini pump (Model 1004, Durect Corporation, Cupertino, CA) was implanted subcutaneously, and then connected via tubing to an ICV cannula (Brain Infusion Kit 3, Durect Corporation, Cupertino, CA). Specifically, the mice were anesthetized using 2% isoflurane and placed in a stereotaxic device. A small midline incision was made to locate bregma. Once located the cannula was placed at +0.02 posterior, -0.95 lateral, and -2.75mm from bregma. The cannula was secured using Loctite 454 (Durect Corporation, Cupertino, CA) and the incision was sutured closed. The osmotic minipumps infused oxytocin (OT) (Bachem, Torrance, CA), selective oxytocin antagonist (OTA) desGly-NH₂-d(CH₂)₅[D-Tyr², Thr⁴]OVT (Manning et al., 2012), or artificial cerebral spinal fluid (aCSF) into the left ventricle of the mouse at a rate of 0.11 $\mu\text{l/hr}$. The total daily dose of OT was 40 or 100ng (based on preliminary studies) and the total daily dose of OTA

was 500ng (Karelina et al., 2011a). The mice in the OT study were singly housed, whereas the mice in the OTA study were group (triad) housed at the time of cannulation. Mice in triad housing were grouped with same-sex, age-matched, ovariectomized familiar Balb/C mice. One week following cannula/osmotic minipump implantation mice received their first dose of vehicle (0.9% saline) or chemotherapeutic cocktail (9 mg/kg doxorubicin and 90 mg/kg cyclophosphamide). Fourteen days later mice received their second injection of vehicle or chemotherapeutic cocktail. Seven days following their second injection mice underwent behavioral testing and tissue collection as previously described for Experiment 2.

2.2 Protein Extraction and Multiplex

Protein Extraction and Multiplex followed a protocol previously described by Walker and coauthors (Walker II et al., 2017). Hippocampi were placed in a 1.5ml tube containing a solution of RIPA buffer (Thermo Fisher Scientific, Waltham, MA) and Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific, Waltham, MA) at a concentration of 1ml/0.1g of tissue and homogenized via sonication. After sonication samples were allowed to incubate on ice for 30 minutes. Next, samples were centrifuged at 13,300 rpm for 15 min at 4°C. The supernatant was removed and placed in a new 1.5 ml tube for subsequent BCA protein assay and Meso Scale Discover V-Plex Pro-inflammatory Mouse Panel. To ensure for equal amount of protein load in each well during protein multiplexing a Pierce BCA Protein Assay (Thermo Fisher Scientific, Waltham, MA) was run according to manufacturer's instructions. To determine cytokine protein concentrations in the serum and hippocampus samples were analyzed using Meso Scale Discover V-Plex Pro-inflammatory Mouse Panel according to manufacturer's instructions. This kit measures protein concentrations of the following cytokines: IFN γ , IL-10, IL-12p70, IL-1 β , IL-2, IL-4, IL-5, IL-6, KC/Gro (CXCL1), and TNF α . The plates were read using a Meso Quickplex machine and the data analyzed via MSD Discovery Workbench software v4.0.

2.3 RNA extraction, cDNA, and qRT-PCR

RNA extraction, cDNA, and qRT-PCR followed a protocol previously described by Walker II et al. (Borniger et al., 2018; Walker II et al., 2017). RNA was extracted using Trizol Reagent (Ambion, Waltham, MA) according to manufacturer's instructions. The quantity and quality of the RNA was measured using a Nanodrop One (Wilmington, DE) spectrophotometer. cDNA was synthesized using M-MLV reverse transcriptase and diluted 1:10. For qRT-PCR 4 μ l (40ng) of diluted cDNA was combined with 16 μ l of master mix solution containing: Taqman Fast Advanced Master Mix (Life Technologies, Carlsbad, CA), an inventoried probe from Applied Biosystems (Life Technologies, Carlsbad, CA) (Fig. S5), a primer-limited probe for the endogenous eukaryotic control 18s rRNA, and water. Each sample was run in duplicate. The qRT-PCR cycling conditions used were 95° C for 20 s, 40 cycles of 95° C for 3 s, and then 60° C for 30 s. Gene expression was quantified using the Pfaffl Method (Pfaffl 2001).

2.4 Immunofluorescence

The paraformaldehyde and sucrose treated brains from were sectioned at 30 μ m into four series using a cryostat. Ionized calcium binding adaptor molecule 1 (Iba1) staining occurred on 30 μ m free floating cryostat sections. Free floating sections were first washed 5 times for

5 min in 1XPBS. Next, sections were placed in a blocking solution containing 1XPBS, 0.1% Triton-X100, and 2.5% NDS for 1 h. Following the one hour block, sections were incubated at room temperature overnight in the primary antibody (rabbit anti-Iba1 1:200, Wako Chemicals, Richmond, VA) plus blocking solution. The following day sections were washed 5 time for 5 minutes in 1XPBS and incubated in the appropriate secondary antibody (594 donkey anti-rabbit, 1:200, Life Technologies Corporation, Eugene, OR) plus blocking solution for 2 h. Next sections were washed again and mounted onto slides and cover slipped with VectaShield + DAPI mounting media (Vector Labs, Burlingame, CA). To analyze Iba1 staining within the hippocampus, five sections located between -1.46 and -2.46 from bregma were randomly chosen. The images were imported into Fiji, the entire hippocampus on these images were outlined and defined as the region of interest (ROI), and the threshold of the image was determined using the default Fiji algorithm. Percent area fraction (i.e., the portion of the region of interest that is above threshold and therefore positively stained) and area of the region of interest were quantified. To account for variability in the size of the region of interest, a weighted average was applied. To calculate the average percent area fraction for each animal, the following formula was used ($\% \text{ Area Fraction of ROI 1} * ((\text{Area of ROI 1})/(\text{Total ROI Area})) + (\% \text{ Area Fraction of ROI 2} * ((\text{Area of ROI 2})/(\text{Total ROI Area})) + (\% \text{ Area Fraction of ROI 3} * ((\text{Area of ROI 3})/(\text{Total ROI Area})) + (\% \text{ Area Fraction of ROI 4} * ((\text{Area of ROI 4})/(\text{Total ROI Area})) + (\% \text{ Area Fraction of ROI 5} * ((\text{Area of ROI 5})/(\text{Total ROI Area})).$

2.5 Behavioral Testing

Mice were tested for novelty-induced locomotor activity via the open field and depressive-like behavior via the forced swim test. All behavioral testing occurred during the dark phase of the light cycle to allow for testing during the active phase of the mouse. Mice were placed for 10 min in a polypropylene open field arena (36 cm x 36 cm) with two rows of infrared sensors mounted on the sides of the box to detect movement (Open Field Photobeam Activity System, San Diego Instruments Inc). Total locomotor activity as well as central tendency were measured and analyzed for each mouse. Following open field testing, mice were transferred to another behavioral room for forced swim test. During forced swim test, mice were placed for 5 min in a 5000ml beaker filled with 3500 ml of water at 27°C. Floating duration, latency to float, and number of floating bouts were recorded and analyzed over the 5 min period. All behavior was scored by an individual that was not aware of group assignment.

2.6 Statistical Analysis

Outliers were defined as having a z score > 2 , and removed prior to any other statistical analysis. At most one data point per group was defined as an outlier. Note, that all experiments were analyzed independently. A one-way ANOVA was used to analyze all data from Experiment 1-Time Course (excluding body mass data; two-tailed t-test). Additionally, a one-way ANOVA was used to examine Experiment 3 - OT Administration (excluding central cytokine data; *a priori* one-tailed t-test). Further, a one-way ANOVA was used to examine Experiment 4- OTA Administration (excluding central cytokine data; *a priori* one-tailed t-test). Post-hoc analysis for experiments 1, 3, and 4 were performed using a Fisher's LSD test. If data failed to meet homogeneity of variance, then a nonparametric Kruskal

Wallis test was performed. Post-hoc analyses on nonparametric data were performed using Dunn's multiple comparisons test. Post-hoc analyses from Experiment 1 only compared each group to the vehicle group as comparisons between all groups during the time course study would not correctly address the hypothesis being studied. A two-way ANOVA was used to analyze all data collected from Experiment 2. Post-hoc analyses were performed using a Fisher's LSD test. If data failed to the assumptions of a two-way ANOVA, then the data were $\log_2$ or square root transformed. If the data failed to meet the assumptions of a two-way ANOVA following transformation then multiple *t*-tests were run, followed by a Bonferroni correction ($p < 0.008$ were considered statistically significant; $(0.05/6 = 0.008)$). Because the protein multiplex measures 10 cytokines simultaneously in the same sample, a correction was made to reduce the likelihood of type 1 error; therefore, a Bonferroni correction was applied and a group difference of $p < 0.005$ ($0.05/10 = 0.005$) was considered statistically significant for data acquired using the Meso Scale Discover V-Plex Pro-inflammatory Mouse Panel. For all other data $p < 0.05$ was considered statistically significant. All statistical analyses were performed using GraphPad Prism 8.0 software.

3. RESULTS

3.1 Experiment 1

Alterations in body mass, spleen mass, and adrenal mass following chemotherapy administration—Mice received two IV injections of vehicle (0.9% saline) or chemotherapeutic cocktail [9 mg/kg doxorubicin (A) and 90 mg/kg cyclophosphamide (C)], at a two week interval. To assess how chemotherapy administration affects the peripheral and central immune response, we collected tissue on days 1, 3, 5, and 7 following the second dose of AC therapy or vehicle. Mice that were administered the chemotherapeutic cocktail displayed significant reductions in body mass on days three ($t = 4.067$, $p < 0.01$), five ($t = 6.481$, $p < 0.001$), and seven ($t = 5.419$, $p < 0.001$) relative to vehicle treated mice. However, no change in body mass was detected on day one post-chemotherapy ($t = 2.036$, $p > 0.05$) (Fig. S1 A). Additionally, chemotherapy administration significantly reduced the spleen mass of mice (Fig. S1 B, $H = 42.07$, $p < 0.001$). Specifically, mice displayed reduced spleen mass on days one ($p < 0.05$, Dunn's test), three ($p < 0.001$, Dunn's test), and five ($p < 0.001$, Dunn's test) relative to vehicle treated mice; there were no significant differences in spleen mass from chemotherapy and vehicle treated groups on day seven ($p > 0.05$ Dunn's test). Adrenal mass was assessed as an index of changes in the chronic activation of the hypothalamus-pituitary adrenal (HPA) axis following chemotherapy administration (Ulrich-Lai et al., 2006); mice receiving doxorubicin and cyclophosphamide displayed increased adrenal masses (Fig. S1 C, $F_{4,50} = 2.564$, $p < 0.01$) on days five ($p < 0.05$, Fisher's LSD) and seven ($p < 0.001$, Fisher's LSD) relative to receiving vehicle.

Chemotherapy administration increases peripheral cytokine production—Serum cytokine concentrations were analyzed following chemotherapeutic administration to determine how peripheral immune responses to chemotherapy change during the week following administration. Mice receiving injections of doxorubicin and cyclophosphamide demonstrated an increase in the concentrations of the following serum cytokines relative to vehicle treated mice: IL-6 (Fig. 1B, $H = 31.68$, $p < 0.001$) (days 1, 3, 5, and 7; $p < 0.01$ for all,

Dunn's test), TNF α (Fig. 1C, $F_{4,52}=17.08$, $p<0.001$) (days 3, 5, and 7; $p<0.01$ for all, Fisher's LSD), IFN γ (Fig. 1D, $H=34.78$, $p<0.001$) (days 1, 3, 5, and 7; $p<0.05$ for all, Dunn's test), IL-2 (Fig. 1E, $H=15.77$, $p<0.005$) (days 1, 3, 5, and 7; $p<0.05$ for all, Dunn's test), CXCL1 (Fig. 1F, $H=38.37$, $p<0.001$) (days 1, 3, 5, and 7; $p<0.01$ for all, Dunn's test), and IL-5 (Fig. 1I, $H=38.66$, $p<0.001$) (days 3, 5, and 7; $p<0.001$ for all, Dunn's test). However, no significant changes in serum cytokine concentrations of IL-1 β (Fig. 1A, $H=10.68$, $p>0.005$), IL-10 (Fig. 1G, $H=4.216$, $p>0.05$), IL-4 (Fig. 1H, $F_{4,32}=1.236$, $p>0.05$), and IL-12p70 (data not shown, $H=6.447$, $p>0.05$) were detected.

Altered central cytokine response following chemotherapy administration—

Concentrations of several cytokines were altered in brain tissue in response to doxorubicin and cyclophosphamide administration. Notably, there were significant reductions in the concentrations of hippocampal IL-4 (Fig. 2F, $F_{4,50}=4.846$, $p<0.005$) and IL-5 (Fig. 2H, $F_{4,50}=8.518$, $p<0.001$) on day three, ($p<0.01$ and $p<0.01$ respectively, Fisher's LSD) relative to vehicle treated mice. Additionally, increases in concentrations of hippocampal cytokines were detected at the later tissue collection time points. Mice administered chemotherapy had increased concentration of the proinflammatory cytokine IL-6 on day seven (Fig. 2B, $F_{4,51}=8.322$, $p<0.001$; $p<0.001$, Fisher's LSD) relative to vehicle treated mice. Further, mice injected with chemotherapy displayed an increase in IL-5 production (Fig. 2H, $F_{4,50}=8.518$, $p<0.001$) on days five ($p<0.01$, Fisher's LSD) and seven ($p<0.05$, Fisher's LSD) relative to vehicle treated mice. No changes in IL-1 β (Fig. 2A, $F_{4,53}=0.7075$, $p>0.05$), TNF α (Fig. 2C, $F_{4,51}=4.091$, $p>0.05$), IFN γ (Fig. 2D, $F_{4,38}=0.1246$, $p>0.05$), CXCL1 (Fig. 2E, $F_{4,52}=2.013$, $p>0.05$) or IL-10 (Fig. 2G, $F_{4,49}=1.307$, $p>0.05$) were detected in the hippocampus. Two cytokines, IL-2 and IL-12p70 failed to reach detectable concentrations within the hippocampus.

3.2 Experiment 2

Social enrichment does not prevent alterations in body mass, spleen mass, and adrenal mass following chemotherapy administration—Next, we sought to determine whether social enrichment could attenuate the elevated peripheral and central pro-inflammatory cytokine signaling seven days following the second injection of doxorubicin and cyclophosphamide. There was a significant effect of both housing and chemotherapy administration on body mass (Fig. S2 A, $F_{1,51}=4.562$, $p<0.05$; $F_{1,51}=131.5$, $p<0.001$). Specifically, mice that were socially isolated (housed individually) or group housed in trios, and administered chemotherapy demonstrated a significant reduction in body mass compared to both vehicle treated groups ($p<0.001$, Fisher's LSD). Additionally, there was a significant effect of chemotherapy treatment on spleen mass (Fig. S2 B, $F_{1,54}=58.99$, $p<0.001$). Mice that were administered chemotherapy displayed a significant decrease in spleen mass compared to vehicle treated mice ($p<0.001$, Fisher's LSD), independent of the type of housing. Further, there was a main effect of chemotherapy treatment on adrenal mass (Fig. S2 C, $F_{1,53}=6.068$, $p<0.05$); specifically, mice that were group housed and administered chemotherapy demonstrated an increase in adrenal mass when compared to group housed, vehicle treated mice ($p<0.05$, Fisher's LSD). However, there were no significant differences when comparing singly housed, chemotherapy treated mice to any other group ($p>0.05$, Fisher's LSD).

Social enrichment alters peripheral cytokine production following

chemotherapy administration—To assess the effects of group housing on peripheral cytokine production, blood was collected via the submandibular vein seven days after the final chemotherapy dose. There was a main effect of chemotherapy on IL-1 β (Fig. 3A, $F_{1,53}=31.53$, $p<0.001$), IL-6 (Fig. 3B, $F_{1,53}=65.43$, $p<0.001$), TNF α (Fig. 3C, $F_{1,53}=66.08$, $p<0.001$), IFN γ (Fig. 3D, $F_{1,55}=74.50$, $p<0.001$), IL-2 (Fig. 3E, $F_{1,51}=53.38$, $p<0.001$), IL-10 (Fig. 3G, $F_{1,53}=9.413$, $p<0.005$), IL-4 (Fig. 3H, $F_{1,37}=9.311$, $p<0.005$), and IL-5 (Fig. 3I, $F_{1,53}=162.7$, $p<0.001$). Specifically, mice that were administered chemotherapy displayed increased IL-1 β (Fig. 3A, $p<0.01$, Fisher's LSD), IL-6 (Fig. 3B, $p<0.001$, Fisher's LSD), IFN γ (Fig. 3D, $p<0.001$, Fisher's LSD), and IL-5 (Fig. 3I, $p<0.001$, Fisher's LSD) when compared to both vehicle treated groups; these effects were independent of housing type. Additionally, there was a significant increase in the concentration of TNF α (Fig. 3C, $p<0.001$, Fisher's LSD) and IL-2 (Fig. 3E, $p<0.005$, Fisher's LSD) in single housed mice that were administered chemotherapy compared to both vehicle groups. Group housing plus chemotherapy administration resulted in an additive effect for TNF α and IL-2 concentrations; group housed mice that were administered chemotherapy had increased concentrations of serum TNF α (Fig. 3C, $p<0.01$, Fisher's LSD) and IL-2 (Fig. 3E, $p<0.01$, Fisher's LSD) compared to all other groups. Notably, the concentrations of the two primary anti-inflammatory cytokines, IL-10 and IL-4, were significantly increased in group housed animals; specifically, IL-10 was increased in group housed animals receiving chemotherapy compared to group housed, vehicle treated mice (Fig. 3G, $p<0.001$, Fisher's LSD). Additionally, group housed mice receiving chemotherapy had significant increased serum IL-4 concentrations compared to all other groups (Fig. 3H, $p<0.05$, Fisher's LSD). One cytokine, CXCL1, was increased specifically in singly housed mice treated with chemotherapy compared to vehicle counterparts (Fig. 3F, $p<0.008$, multiple t -tests Bonferroni correction). No changes were detected in the serum concentration of IL-12p70 (data not shown, $p>0.05$).

Social enrichment attenuates central cytokine signaling and depressive-like behavior following chemotherapy administration

—Next, we sought to determine the effects of social enrichment on central cytokine signaling following chemotherapy administration. One randomly selected hemisphere was processed for PCR, whereas the other hemisphere was processed for histology. We first measured the mRNA expression of the pro-inflammatory cytokines *il-1 β* , *il-6*, and *tnfa*. There was a main effect of chemotherapy treatment on the expression of *il-1 β* and *il-6* within the hippocampus (Fig. 4A-B, $F_{1,51}=4.553$, $p<0.05$; $F_{1,53}=4.125$, $p<0.05$). Specifically, mice that were social isolated and administered chemotherapy displayed increased *il-1 β* and *il-6* expression compared to both vehicle groups (Fig. 4A-B, $p<0.05$, Fisher's LSD). Notably, group housed mice that were administered chemotherapy demonstrated an attenuation of *il-1 β* and *il-6* expression in the hippocampus; group housed, chemotherapy treated, mice did not significantly differ in the expression of *il-1 β* or *il-6* when compared to either vehicle treated group ($p>0.05$, Fisher's LSD). Additionally, there was a significant interaction in the expression of *tnfa* (Fig. 4C, $F_{1,53}=7.181$, $p<0.05$); specifically, mice that were group housed and vehicle treated demonstrated reduced *tnfa* relative to singly housed vehicle treated mice ($p<0.05$, Fisher's LSD). To determine whether increased cytokine expression

within the hippocampus corresponded to an increase in microglia activation, percent area fraction of Iba1 staining within the hippocampus was measured; there was no effect of housing or chemotherapy treatment on Iba1 staining (Fig. 4D, $F_{1,45} = 2.108$; $F_{1,45} = 2.050$, $p > 0.05$). Additionally, we sought to assess whether the attenuation in *il-1 β* and *il-6* expression within the hippocampus in chemotherapy treated group housed mice was functionally significant. Mice were tested in two behavioral tasks, forced swim test, a measure of depressive-like behavior, and open field test, a measure of exploratory behavior. There was a main effect of chemotherapy treatment on floating duration (Fig. 4, $F_{1,64} = 5.154$, $p < 0.05$). Specifically, singly housed mice that were treated with chemotherapy increased floating duration when compared to singly housed, vehicle treated mice ($p < 0.05$, Fisher's LSD). Notably, there was an attenuation of floating duration in chemotherapy treated group housed animals. However, there was no effect of housing or chemotherapy treatment on the number of floating bouts (Fig. 4F, $F_{1,66} = 0.2665$; $F_{1,65} = 0.1148$, $p > 0.05$) or latency to float (Fig. 4G, $F_{1,65} = 0.9974$; $F_{1,65} = 3.401$, $p > 0.05$). Together, these data suggest increased depressive-like behavior in socially isolated, chemotherapy treated mice. Additionally, these effects are attenuated by social enrichment. To determine the effects of chemotherapy administration on locomotor activity behavior in a novel environment, mice were tested in an open field apparatus. There was a significant effect of chemotherapy treatment (Fig. 4H, $F_{1,67} = 33.02$, $p < 0.001$) and housing (Fig. 4H, $F_{1,67} = 8.855$, $p < 0.01$) on total beam breaks. Mice that were vehicle treated and singly housed had increased locomotor activity compared to all other groups ($p < 0.01$, Fisher's LSD). Additionally, mice that received chemotherapy treatment and were group housed demonstrated a significant reduction in locomotor activity in the novel environment when compared to vehicle treated pair housed mice ($p < 0.01$, Fisher's LSD). Further, there was a main effect of chemotherapy treatment on central tendency (Fig. 4I, $F_{1,64} = 7.659$, $p < 0.01$); specifically, mice that were treated with chemotherapy displayed reduced central tendency independent of the type of housing when compared to group housed, vehicle treated mice ($p < 0.05$, Fisher's LSD). These results are suggestive of decreased exploratory behavior in chemotherapy treated mice.

3.3 Experiment 3

Administration of oxytocin to socially isolated mice ameliorates pro-inflammatory cytokine production and depressive-like effects after chemotherapy—Because social housing increases oxytocin expression within the brain and oxytocin has demonstrated effects in reducing pro-inflammatory cytokine production and improving affective behavior (Karelina et al., 2011; Karelina and Devries, 2011; Karelina and Norman, 2009; Norman et al., 2010a), exogenous oxytocin was administered to singly housed mice to determine whether oxytocin may be responsible for observed reduction in pro-inflammatory cytokines and depressive-like behavior in group housed mice treated with chemotherapy. Exogenous oxytocin administration to singly housed, chemotherapy treated mice had no effect on previously reported changes body mass, spleen mass, or adrenal mass (Fig. S3). There was a significant effect of chemotherapy treatment on the number of floating bouts in the swim test (Fig. 5B, $F_{3,53} = 4.302$, $p < 0.05$); additionally, planned comparisons showed statistically significant difference in floating duration, the overall ANOVA for the effects of chemotherapy treatment on floating duration was not

significant (Fig. 5A, $F_{3,52} = 2.548$, $p = 0.06$). Specifically, singly housed, chemotherapy treated mice that were administered 100ng OT daily had reduced number of floating bouts and floating durations compared to singly housed, chemotherapy treated mice receiving aCSF vehicle ($p < 0.05$, Fisher's LSD). Notably, the number of floating bouts and floating duration of the singly housed, chemotherapy treated mice that were administered 100ng OT did not significantly differ from singly housed, vehicle treated mice that received aCSF ($p > 0.05$, Fisher's LSD). Mice that were administered 40ng of OT daily and treated with chemotherapy demonstrated an intermediate effect. No main effect of chemotherapy treatment was seen in latency to float (Fig. 5C, $p > 0.05$). There was a significant effect of chemotherapy treatment on total beam breaks in the open field (Fig. 5D, $F_{3,53} = 2.903$, $p < 0.05$). Mice that were administered 40ng OT daily had increased activity compared to singly housed, chemotherapy treated mice receiving aCSF ($p < 0.05$, Fisher's LSD). Mice that were administered 100ng of OT daily and treated with chemotherapy demonstrated an intermediate effect. To determine whether the beneficial effects of oxytocin were mediated via reduced pro-inflammatory cytokine expression, we assessed the expression of three primary pro-inflammatory cytokines within the hippocampus; we utilized 100ng OT treatment specifically as it reduced depressive-like behavior (Fig 5A-B). Singly housed, chemotherapy treated mice that received 100ng of OT daily had reduced expression of *il-6* within the hippocampus relative to singly housed, chemotherapy treated mice receiving aCSF (Fig. 5G, $p < 0.05$; *a priori* one-tailed *t*-test). There was no effect on the expression of *il-1 β* or *tnfa* within the hippocampus (Fig. 5F and H). Together, these data suggest that the beneficial effects of OT treatment may be mediated via suppressed *il-6* within the hippocampus.

3.4 Experiment 4

Administration of oxytocin antagonist to group housed mice increases pro-inflammatory and depressive-like effects of chemotherapy—To further examine a potential protective role of oxytocin among group housed mice, an oxytocin receptor antagonist or vehicle was administered to grouped housed, chemotherapy treated mice and central cytokine signaling and affective behavior were assessed. Exogenous oxytocin antagonist administration to triad housed, chemotherapy treated mice had no effect on previously reported changes in body mass, spleen mass, or adrenal mass (Fig. S4). There was a significant effect of chemotherapy treatment on floating duration, number of floating bouts, and latency to float in the swim test. Specifically, pair housed mice that received chemotherapy and oxytocin antagonist demonstrated significantly longer floating duration (Fig. 5I, $F_{2,32} = 3.406$, $p < 0.05$), increased number of floating bouts (Fig. 5J, $F_{2,32} = 11.73$, $p < 0.001$), and decreased latency to float (Fig. 5K, $F_{2,32} = 4.827$, $p < 0.05$) when compared to all other groups ($p < 0.05$, Fisher's LSD). There was no effect of treatment on total locomotor activity or central tendency (Fig 5 L-M). To provide further evidence that the beneficial effects of group housing may be oxytocin-mediated via reduced IL-6, we assessed central cytokine production among group housed, OTA treated mice. Mice that were group housed, chemotherapy treated and received OTA had increased *il-6* expression within the hippocampus relative to group housed, chemotherapy treated mice receiving aCSF (Fig. 5O, $p < 0.05$; *a priori* one-tailed *t*-test). There was no effect on the expression of *il-1 β* or *tnfa* within the hippocampus (Fig. 5N and P). Together, these data demonstrate an increased

depressive-like phenotype in the chemotherapy treated, group housed mice that received oxytocin antagonist, thereby recapitulating the effect of chemotherapy on singly housed mice. Additionally, these data provide further evidence that the beneficial effects of oxytocin may be mediated via suppressed *il-6* within the hippocampus.

4. DISCUSSION

The affective side effects of chemotherapy treatment reduce the quality of life of breast cancer survivors and are associated with reduced survival, as such determining the underlying mechanisms is a critical first step in optimizing treatment. The data presented here demonstrate that following the second treatment of dose-dense doxorubicin and cyclophosphamide therapy there is increased peripheral and central pro-inflammatory cytokine signaling and increased depressive-like behavior in mice. Furthermore, social enrichment, achieved through group housing, resulted in an attenuation of central pro-inflammatory cytokine signaling and depressive-like behavior following AC therapy. Additionally, central administration of oxytocin to socially isolated animals treated with chemotherapy ameliorated *il-6* expression within the hippocampus and depressive-like behavior. Further, administration of oxytocin receptor antagonist to group housed, chemotherapy-treated animals recapitulated the increased depressive-like behavior and hippocampal *il-6* previously observed in singly housed animals following AC administration. These results demonstrate a potential role for endogenous oxytocin in the protective effects of social enrichment, which may be mediated via IL-6 signaling.

First, we investigated a time course of changes in peripheral and central cytokine concentrations during the week following the second administration of AC therapy. We sought to determine how the previously reported acute (24 h) biphasic cytokine response after treatment with chemotherapy changed over the week following chemotherapy administration (Borniger et al., 2017; Smith et al., 2014). Mice that were administered chemotherapy had increased peripheral pro-inflammatory cytokine signaling (IL-6, IFN γ , IL-2, CXCL1) as early as 24 h following chemotherapy injections (Fig. 2). However, no biphasic response was detected. The peripheral measures of cytokine concentrations remained elevated throughout the study, with all major pro-inflammatory cytokines (except IL-1 β) exhibiting significant increases in the serum concentrations through day seven. Notably, serum concentrations of two primary anti-inflammatory cytokines, IL-10 and IL-4 did not change across time or relative to the vehicle. Taken together, these serum data suggest a highly pro-inflammatory Th1/M1 response following AC treatment (Mosmann and Sad, 1996; Wang et al., 2014).

Concentrations of central (hippocampal) cytokines did not mirror the peripheral effects of AC treatment. There were no significant alterations in hippocampal cytokine concentrations until day three (Fig.2), at which point, mice administered doxorubicin and cyclophosphamide had significant reductions in the anti-inflammatory cytokines IL-4 and IL-5 relative to the vehicle group. Reduced IL-4 concentrations in the brain have previously been associated with increased central pro-inflammatory cytokine production and cognitive deficits (Derecki et al., 2010; Gadani et al., 2012). Central IL-4 production reduces pro-inflammatory cytokine signaling and supports repair by promoting an anti-inflammatory

(M2) phenotype in microglia and increasing production of brain derived neurotrophic factor by astrocytes (Gadani et al., 2012). Further, mice lacking IL-4 production from meningeal T cells exhibit deficits in learning and memory (Derecki et al., 2010). Thus, while not explicitly tested, one might predict that transient IL-4 related alterations in behavior, such as cognitive function, on day 4. In the present study, however, the reductions in IL-4 and IL-5 are unlikely to explain the alterations in central pro-inflammatory cytokine production and depressive-like behavior as these reductions resolve by day 5. In addition to the reductions in IL-4 and IL-5 concentrations in the hippocampus following AC therapy, there was a significant elevation in the pro-inflammatory cytokine IL-6 one week after AC treatment (Fig. 2). Previous studies investigating the acute effects of chemotherapy treatment on the brain, have demonstrated similar increases in pro-inflammatory cytokine levels following chemotherapy administration that are associated with deficits in fatigue, cognitive function, and sleep (Borniger et al., 2015; Borniger et al., 2017; Shi et al., 2019; Weymann et al., 2014). However, to our knowledge this is the first time that increased pro-inflammatory cytokine concentrations within the brain have been demonstrated one week following AC treatment.

Because of the demonstrated beneficial effects of social enrichment on several disease outcomes (Craft et al., 2005; Karelina and Devries, 2011; Maunsell et al., 1995; Norman et al., 2010; Norman et al., 2010b; Verma et al., 2014; Waxler-Morrison et al., 1991), we examined whether social enrichment (triad housing) could attenuate the elevated peripheral and central cytokine signaling following chemotherapy administration. Although social enrichment significantly increased the concentrations of the pro-inflammatory cytokines IL-2 and TNF α , it also produced significant increases in concentrations of anti-inflammatory cytokines IL-10 and IL-4, both of which are markers for Th2/M2 phenotype. Indeed, IL-4 production alone is sufficient to induce all Th2 effector functions and redirect macrophages to an M2 (anti-inflammatory) phenotype (Biswas and Mantovani, 2010; Fallon et al., 2002). Although not directly tested, these data suggest social enrichment may produce beneficial effects on peripheral cytokine signaling by reducing the Th1/M1 response observed in singly housed animals following AC treatment.

The beneficial effects of social enrichment were not limited to the periphery. Group housed mice displayed significant reductions in hippocampal *il-1 β* and *il-6* expression (Fig. 4). Similar social enrichment-induced reductions in pro-inflammatory cytokine expression have been reported following peripheral nerve injury and stroke (Norman et al., 2010; Verma et al., 2014). Chemotherapy administration did not have a significant effect on the expression of *tnfa* mRNA in the hippocampus among either singly or group-housed mice. Further, even with the increase in *il-1 β* and *il-6* mRNA expression, no changes were detected in Iba1 percent area fraction (an index of microglial activation) within the hippocampus (Fig. 4), suggesting that either (1) a different population of cells are secreting *il-1 β* and *il-6* following AC administration (Allan et al., 2005; Gruol, 2015; Vallières et al., 2002) or (2) microglia may still be involved and Iba1 percent area fraction is not sufficiently sensitive to detect subtle microglial activation; thus alternative approaches (i.e. RNAseq) may be required to more confidently rule out microglial activation.

Because patients receiving chemotherapy frequently display increases in anxiety and depression (Hipkins et al., 2004; Pandey et al., 2006; Saevarsdottir et al., 2010), and proposed explanations for these deficits include increased central pro-inflammatory cytokine production (Seigers and Fardell, 2011), we next sought to assess the effects of chemotherapy and social enrichment on depressive-like (forced swim test) and exploratory (open field test) behavioral responses. Singly housed mice that received chemotherapy increased floating duration relative to singly housed, vehicle treated mice, which is indicative of increased depressive-like behavior. Notably, group housed mice had reduced depressive-like behavior following AC treatment relative to socially isolated mice (Fig. 4H). The protective effects of social enrichment on depressive-like behavior are not limited to chemotherapy-induced increases in depressive-like behavior, as social enrichment also has been demonstrated to prevent the development of depressive-like behavior post nerve injury, cardiac arrest, and stroke (Norman et al., 2010a, 2010b; Verma et al., 2014). Additionally, mice administered chemotherapy had significantly decreased locomotor activity, independent of housing (Fig. 4E). Typically, reduced locomotor activity following chemotherapy is interpreted as an indication of fatigue (Weymann et al., 2014). In agreement with previous studies (Merzoug et al., 2011), mice receiving chemotherapy displayed significant reductions in central tendency, when compared to pair housed, vehicle treated mice (Fig. 4). Together, these data demonstrate increased depressive-like behavior in mice receiving chemotherapy. Group housing mice results in the attenuation of depressive-like behavior following AC treatment. Future studies should examine the effects of chemotherapy plus social enrichment in other tests of depressive-like behavior such as sucrose place preference or sucrose slash test as these tasks assesses the motivation of mice to engage in pleasure seeking behavior, but requires less energy expenditure than the forced swim test (Amini-Khoei et al., 2017).

The neuroprotective effects of social enrichment have previously been associated with increased oxytocin signaling within the brain (Karelina et al., 2011; Smith and Wang, 2012). Therefore, we sought to determine whether oxytocin administration to socially isolated, chemotherapy-treated mice could ameliorate the pro-inflammatory cytokine production and depressive-like effects of chemotherapy treatment. Indeed, ICV infusion of 100ng OT daily to singly housed, chemotherapy treated mice reduced floating duration and number of floating bouts during the forced swim test and prevented increased hippocampal *il-6* expression following AC therapy (Fig. 5); these results suggest that the protective effects of social enrichment may be mediated via the ability of oxytocin to suppress IL-6 signaling within the brain. Indeed, previous studies have demonstrated the immunomodulatory properties of oxytocin (Amini-Khoei et al., 2017; Karelina et al., 2011; Szeto et al., 2008). Specifically, these studies have demonstrated that oxytocin can suppress peripheral IL-6 expression and simultaneously reduce the activation of macrophages, endothelial cells, and microglia (Karelina et al., 2011; Szeto et al., 2008). To provide further evidence that the protective effects of social enrichment may be OT/IL-6 mediated, we administered OTA to group housed, chemotherapy treated mice. Indeed, mice that were group housed and received chemotherapy treatment, as well as a constant ICV infusion of oxytocin antagonist (500ng daily), increased floating duration, increased floating bouts, and decreased latency to float compared to all other groups (Fig. 5); thus, demonstrating increased depressive-like behavior in mice receiving AC treatment plus oxytocin receptor antagonist. Additionally,

administration of chemotherapy plus the oxytocin antagonist to group housed animals increased hippocampal *il-6* expression (Fig. 5). Taken together, these data suggest that the beneficial effects of social enrichment following AC administration may be mediated via oxytocin, and based on prior literature may be related to its ability to suppress IL-6 signaling within the brain. Oxytocin receptors are expressed on neurons within the hippocampus and are present in microglia, monocytes, and other lymphocytes (Gimpl and Fahrenholz, 2001; Raam et al., 2017; Szeto et al., 2017; Yuan et al., 2016). Thus, we would propose that the immunomodulatory effects of oxytocin could be mediated via direct action of oxytocin signaling on immune cells. Because the focus of the study was specifically on whether social housing may alter the cytokine and behavioral responses to chemotherapy via an OT-related mechanism, the present study did not include singly housed, vehicle treated mice receiving OT or socially housed vehicle treated mice receiving OT antagonist. These groups were not included because they increased the number of animals required for the study without clarifying whether socially-induced variation in OT signaling could underlie the observed differences in IL-6 and behavior between socially isolated versus group housed mice following treatment with AC chemotherapy. However, providing exogenous OT to singly housed, vehicle treated mice or OTA to triad-housed vehicle treated mice would have provided additional information on whether modulating OT signaling has effects on cytokine expression or behavior in the absence of an inflammatory stimulus, such as chemotherapy. The existing literature does not provide strong evidence for such an effect. For example, in rats, OT and OTA administered to otherwise unmanipulated animals did not alter affective behavior until administered at high pharmacological doses (i.e. >500 times our daily dose) (Yan et al., 2014). Likewise, neither acute nor chronic OT administration altered depressive-like behavior in Wistar rats selectively bred for anxiety-like responses (Slattery and Neumann, 2010). Additionally, exogenous OT reduces bacterial endotoxin-induced, but not basal, IL-6 expression in human males (Clodi et al., 2008). In sum, the current study specifically examines a potential role for OT in social modulation of responses to AC chemotherapy; it indicates that exogenously amplifying OT in socially isolated mice treated with chemotherapy reduces IL-6 and behavioral deficits, while reducing OT signaling among group housed mice with an OT receptor antagonist increases IL-6 and behavioral deficits. Additional studies are needed to clarify the extent to which OT modulates depressive-like behavior and immune function under basal conditions.

Notably, patients receiving chemotherapeutics frequently display elevated IL-6 concentrations within the blood (Liu et al., 2012; Penson et al., 2000; Pusztai et al., 2004), and this increase in IL-6 signaling is frequently correlated with reduced cognitive function and increased depression, fatigue, and sleep disturbances (Kesler et al., 2013; Liu et al., 2012; Lutgendorf et al., 2008; Wang et al., 2012). These effects are remarkably consistent in foundational science research (Borniger et al., 2015; Shi et al., 2019; Weymann et al., 2014). Indeed, our lab has previously demonstrated that a single dose of AC therapy to non-tumor bearing mice increased NREM and REM sleep with a concurrent increase in sleep fragmentation (Borniger et al., 2015). These deficits were positive correlated with IL-6 expression within the hippocampus. Thus, future studies will examine the effects of social enrichment on sleep disturbances and cognitive dysfunction following chemotherapy administration.

Limitations and Future Directions

While every effort was made to design the present study to model social support or lack thereof in breast cancer survivors receiving chemotherapy treatment, there are, as in all studies, limitations. First, the present study focused specifically on the effects of chemotherapy induced pro-inflammatory cytokine production and affective behavior. However, treatment for breast cancer survivors expands beyond chemotherapy treatment. Future studies should examine the behavioral effects of anti-hormonal therapies, radiation therapies, and anti-angiogenic therapies that are often paired with chemotherapy in the treatment of breast cancer. Additionally, the present study used tumor-free mice, which may not reflect tumor-bearing patients receiving chemotherapy. This approach was used as it allowed for investigation and attribution of the effects of chemotherapy alone. Thus, future studies should examine these effects in tumor bearing mice. Further, in an attempt to control for the confound of acute ovarian failure in response to chemotherapy, mice underwent an ovariectomy procedure. Studies have demonstrated a potential priming effect of surgery on central cytokine production and behavioral deficits (Hovens et al., 2012). While immune priming cannot definitively be ruled out as it was not explicitly tested within the present study, it is not likely to explain the increased *il-6* expression within the hippocampus following chemotherapy administration, due to the fact that increased cytokine signaling did not occur within the brain until seven days following chemotherapy administration and was specific to *il-6* and was not observed in other pro-inflammatory cytokines. Furthermore, throughout the study, mice were triad housed one week prior to the onset of chemotherapy treatment, which may not reflect a potentially more stable (i.e. long-lasting) social support network; however, participation in group activities postdiagnosis reduced anxiety and depression in breast cancer patients, suggesting that short-term social support may be beneficial (Kroenke et al., 2006; Montazeri et al., 2001). Finally, while the present study used forced swim test as a measure of depressive-like behavior, other authors have argued against this use of this behavioral task (Molendijk and de Kloet, 2019; Molendijk and Ronald de Kloet, 2015). They argue that floating in the forced swim test is an adaptive response that reflects a switch from active to passive coping strategies. Thus, (1) given the increases in IL-6 in the present study and (2) that increases in IL-6 signaling in cancer patients has been correlated with fatigue, among other behavioral alterations (Kesler et al., 2013; Liu et al., 2012; Lutgendorf et al., 2008; Wang et al., 2012), an alternative interpretation of the results could be that singly housed mice receiving chemotherapy have increased sickness behavior/fatigue and the increased immobility during the forced swim test represents an alternative coping strategy. Furthermore, due to the attenuation in immobility and potential increase in Th2/M2 response following AC treatment in group housed mice, social enrichment may instead promote stress-coping and healing.

5. CONCLUSIONS

In summary, these data demonstrate increased peripheral and central pro-inflammatory cytokine signaling and increased depressive-like behavior following the second cycle of dose-dense AC therapy in mice. Social enrichment throughout chemotherapy treatment lead to an attenuation of central pro-inflammatory cytokine signaling and depressive-like behavior. Additionally, social enrichment increased the production of anti-inflammatory

cytokines within the periphery, suggesting a potential increase in Th2/M2 response following AC treatment in group housed mice. Administration of exogenous oxytocin to socially isolated mice ameliorated the depressive-like behavior and reduced *il-6* expression within the hippocampus. Administration of oxytocin antagonist to group housed mice receiving chemotherapy recapitulated the increased depressive-like behavior and hippocampal *il-6* expression previously reported in singly housed mice receiving AC treatment; these results suggest a potential role for oxytocin in the protective effects of social enrichment via a suppression of IL-6 signaling within the brain. Ultimately, these data add to the growing literature detailing the negative side effects of chemotherapy and provide further evidence that social enrichment may be beneficial in offsetting some of the negative side effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

The authors acknowledge the excellent care provided to the animals used in these studies from the WVU Animal Resources personnel. We further acknowledge Cornelius Braam, Abigail Zalenski, Stevie Muscarella, Julie Fitzgerald, and Tial TinKai for their assistance and Randy J. Nelson for his comments. The authors were supported by grants from NCI (R01CA194924 ACD) and NIGMS under award number 5U54GM104942-03. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Highlights

- Chemotherapy increases IL-6 within the brain and alters depressive-like behavior.
- Social housing attenuates chemotherapy-induced CNS alterations.
- Oxytocin administration ameliorates IL-6 expression and depressive-like behavior.
- Oxytocin antagonist administration increases IL-6 expression and depressive-like behavior.

Figure 1.

Chemotherapy administration increases peripheral cytokine production. Mice administered chemotherapy had increased concentrations of (B) IL-6 (days 1, 3, 5, 7), (C) TNF α (days 3, 5, 7), (D) IFN γ (days 1, 3, 5, 7), (E) IL-2 (days 1, 3, 5, 7), (F) CXCL1 (days 1, 3, 5, 7), and (I) IL-5 (days 3, 5, 7) in the serum following the second injection of doxorubicin and cyclophosphamide. There were no changes in the serum concentrations of (A) IL-1 β , (G) IL-10, and (H) IL-4. (n= 18-19 for vehicle and 8-10 per group for chemo for all except IL-2 n= 15 for veh n= 8-10 for chemo and IL-4 n= 8 for vehicle and n= 5-9 for chemo) The vehicle group represents a combination of data collected across days 1, 3, 5, and 7; prior to combining data, statistical analyses were run to ensure equivalent concentrations ($p > 0.05$) across days within the vehicle group. The data are presented as mean $\pm$ SEM. * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ **** $p < 0.0001$.

Figure 2.

Variable central cytokine response following chemotherapy administration. Mice administered chemotherapy demonstrated reduced hippocampal concentrations of (F) IL-4 and (H) IL-5 on day three following the second chemotherapy injection. Additionally, there was a significant increase in the concentration of hippocampal (H) IL-5 (days 5 and 7) and (B) IL-6 (day 7). There were no significant group differences in hippocampal concentrations of (A) IL-1 β , (C) TNF α , (D) IFN γ , (E) CXCL1, and (G) IL-10. (n= 16-19 for vehicle and 8-10 per group for chemo for all except IFN γ n= 12 for vehicle n= 7-8 for chemo). The vehicle group represents a combination of data collected across days 1, 3, 5, and 7; prior to combining data, statistical analyses were run to ensure equivalent concentrations (p>0.05) across days within the vehicle group. The data are presented as mean +SEM. * p<0.05 ** p<0.01 *** p<0.001 **** p<0.0001. # Main effect of chemotherapy p<0.005.

Figure 3.

Social enrichment alters serum cytokine production following chemotherapy administration. Mice administered chemotherapy had increased serum concentrations of (A) IL-1 β , (B) IL-6, (C) TNF α , (D) IFN γ , (E) IL-2 and (I) IL-5 independent of housing treatment; group housing plus chemotherapy displayed a synergistic effect on the concentration of TNF α and IL-2 within the serum. Mice that were single housed and treated with chemotherapy had a significant increase in (F) CXCL1 when compared to singly housed, vehicle treated mice; group housed, chemotherapy treated mice displayed an attenuated concentration of CXCL1. Additionally, group housed mice that received chemotherapy displayed increases in the concentrations of anti-inflammatory cytokines (H) IL-4 and (G) IL-10. (n=13-15 per group except IL-2 n= 11-15 and IL-4 n= 7-15) The data are presented as mean +SEM. Graph bars that do not share a letter are statistically significant different at p<0.05. # Main effect of chemotherapy, \$ Main effect of housing, and + chemotherapy X housing effect at p<0.005.

Figure 4.

Social enrichment attenuates central cytokine signaling and behavioral deficits following AC therapy. Single housed mice treated with chemotherapy had a significant increase in hippocampal (A) *il-1 β* and (B) *il-6* mRNA expression when compared to singly housed, vehicle treated mice; pair housing attenuated the chemotherapy induced increase in *il-1 β* and *il-6* mRNA expression. There was a significant interaction in (C) *tnfa* mRNA expression; mice that were group housed and vehicle treated demonstrated reduced *tnfa* expression when compared to single housed vehicle treated mice. There were no significant effects of housing or chemotherapy treatment on (D) Iba1, a marker of microglial activation. Singly housed mice that were administered chemotherapy demonstrated increased (E) floating duration when compared to singly housed, vehicle treated mice respectively; group housing attenuated the increase in floating duration seen in chemotherapy treated mice. No significant differences were detected in the (G) latency to float or (F) number of floating bouts. Additionally, mice treated with chemotherapy had reduced (H) novel locomotor activity compared to singly housed, vehicle treated mice; group housed mice treated with chemotherapy displayed a further reduction in total locomotor activity when compared to group housed, vehicle treated mice. There was a significant reduction in (I) central tendency of mice treated with chemotherapy when compared to group housed, vehicle treated mice. The data are presented as mean $\pm$ SEM. (n=12-15 per group A-D) (n=16-18 per group E-I) Graph bars that do not share a letter are statistically significant different at $p < 0.05$. # Main effect of chemotherapy \$ Main effect of housing + chemotherapy X housing effect at $p < 0.05$.

Figure 5.

Administration of an oxytocin to singly housed animals ameliorates *il-6* expression and depressive-like effects of chemotherapy. Whereas, administration of an oxytocin antagonist to group housed animals induces *il-6* expression and depressive-like effect of chemotherapy. Mice that received chemotherapy plus oxytocin (100ng) had a reduced (A) floating duration and (B) number of floating bouts, when compared to singly housed chemotherapy treated mice; there was no significant difference when compared to singly housed vehicle treated mice. Thus, demonstrating a reduced depressive-like behavior for mice that received chemotherapy plus oxytocin (100ng). Mice receiving chemotherapy plus oxytocin (40ng) demonstrated an intermediate effect. Singly housed chemotherapy treated mice displayed reduced (D) locomotor activity; administration of oxytocin (40 or 100ng) attenuated the reduction in locomotor activity. No changes were detected in (C) latency to float or (E) central tendency. Additionally, mice that received chemotherapy plus oxytocin (100ng) demonstrated reduced hippocampal *il-6* mRNA expression (G). No changes were detected in hippocampal *il-1 β* or *tnfa* expression (F and H). Mice that received chemotherapy plus oxytocin antagonist treatment displayed increased floating duration (I), number of floating

bouts (J), and decreased latency to float (K) when compared to all other groups; demonstrating an increase in depressive-like behavior for these mice. No changes were detected in total beam breaks (L) or central tendency (M). Additionally, chemotherapy treated group housed mice administered oxytocin antagonist demonstrated increased hippocampal *il-6* expression (O). No changes were detected in hippocampal *il-1 β* or *tnfa* expression (N and P). The data are presented as mean +SEM. (n=13-15 A-E) (n=7-9 F-H) (n=11-13 per group I-M) (n=8-9 per group N-P). Graph bars that do not share a letter are statistically significant different at $p<0.05$. # Main effect of treatment. * $p<0.05$.

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