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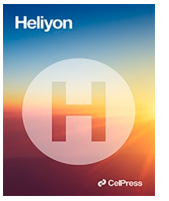

Social enrichment alters the response of brain leukocytes to chemotherapy and tumor development in aged mice

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ABSTRACT

Aging is a risk factor for the development of breast cancer. Foundational science studies have supported associations among neuroinflammation, breast cancer, and chemotherapy, but to date, these associations are based on studies using young adult rodents. The current study examined the neuroinflammatory effects of chemotherapy in aged, tumor-naïve and tumor-bearing mice with or without social enrichment. Mice received two intravenous injections of doxorubicin (A) and cyclophosphamide (C) at a two-week interval. Brain immune cells were enriched/assessed via flow cytometry, seven days following the second chemotherapy injection. Social enrichment enhanced peripheral immune cell trafficking in aged tumor-naive mice treated with AC. Group housed aged tumor bearing mice receiving AC had reduced percentage of IL-6+ monocytes and granulocytes relative to their singly housed counterparts. Notably, group housing aged experimental mice with young cage partners significantly reduced TNF + monocytes, tumor volume, and tumor mass. These data illustrate the importance of social enrichment in attenuating neuroinflammation and are the first to demonstrate that social support with young housing partners reduces tumor growth in aged mice.

1. Introduction

Breast cancer affects large numbers of women annually. In 2019, the latest year for which incidence data are currently available, the CDC reported the incidence rate of female breast cancer as 130 cases per 100,000 women, making breast cancer the leading cause of cancer diagnoses in women. Breast cancer patients frequently report altered sleep, affective behaviors, and cognition [1–4]. Foundational science studies have supported these associations, as mammary tumor bearing mice display alterations in sleep, the development of depressive-like behavior, and cognitive impairment [5–8]. Notably, these relationships are reciprocal. Clinically, there are also established relationships among altered sleep, cognitive deficits, development of depression/anxiety, and cancer progression/cancer survival [4,9–11]. Indeed, women reporting 6 h or less of sleep at cancer diagnoses are more likely to have tumors staged as regional or distant [11]. A recent meta-analysis of over 280,000 breast cancer patients reports a significant association

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between depression and cancer reoccurrence and depression and cancer-specific mortality [4]. Additionally, a recent retrospective study of older (>67 years) female patients diagnosed with breast cancer demonstrate a significant association between cognitive impairments and cancer-specific mortality [9].

Chemotherapy treatment is associated with worsening of cognitive deficits, sleep alterations, and development of depression in breast cancer patients [12–15]. In a cohort of ~70 women with stage I-III breast cancer who received ≥4 cycles of chemotherapy, patients exhibited worse sleep and more depressive symptoms following their 4th cycle of chemotherapy relative to their baseline and cancer-free controls [12]. Additionally, a meta-analysis comprised of 13 studies (997 subjects) examined the effects of chemotherapy on seven different cognitive domains, across five cancer types and reported a significant negative correlation between chemotherapy treatment duration and cognitive impairment [16].

In the absence of cancer, mice receiving chemotherapy exhibit altered sleep, increased depressive-like behavior, and cognitive deficits, suggesting that chemotherapy treatment alone is sufficient to alter behavior [17–21]. The precise neurobiological basis of chemotherapy induced alterations in sleep, cognitive function, and depression remains unspecified. However, common hypotheses include neuroinflammation, oxidative stress, decreased blood flow, decreased neurotrophic factors, and decreased neurogenesis [3, 22–26]. Overall, the balance of empirical evidence supports the hypothesis that neuroinflammation is responsible for the neuropsychological and sleep effects of chemotherapy [27].

Social support is associated with both the development of behavioral changes in breast cancer patients and cancer progression/mortality [28–32]. Patients with low social support prior to treatment report increased levels of pain and depressive symptoms relative to patients with robust social support [28]. Likewise, social support is negatively correlated with depression and anxiety in a cohort of 220 breast cancer patients [32]. Short-term social support also may be beneficial, as breast cancer patients who participate in group activities post-diagnosis exhibit reduced anxiety and depression [33,34]. In addition to influencing quality of life, there are compelling data to suggest that social interactions influence disease course. Social isolation prior to breast cancer diagnoses is associated with a

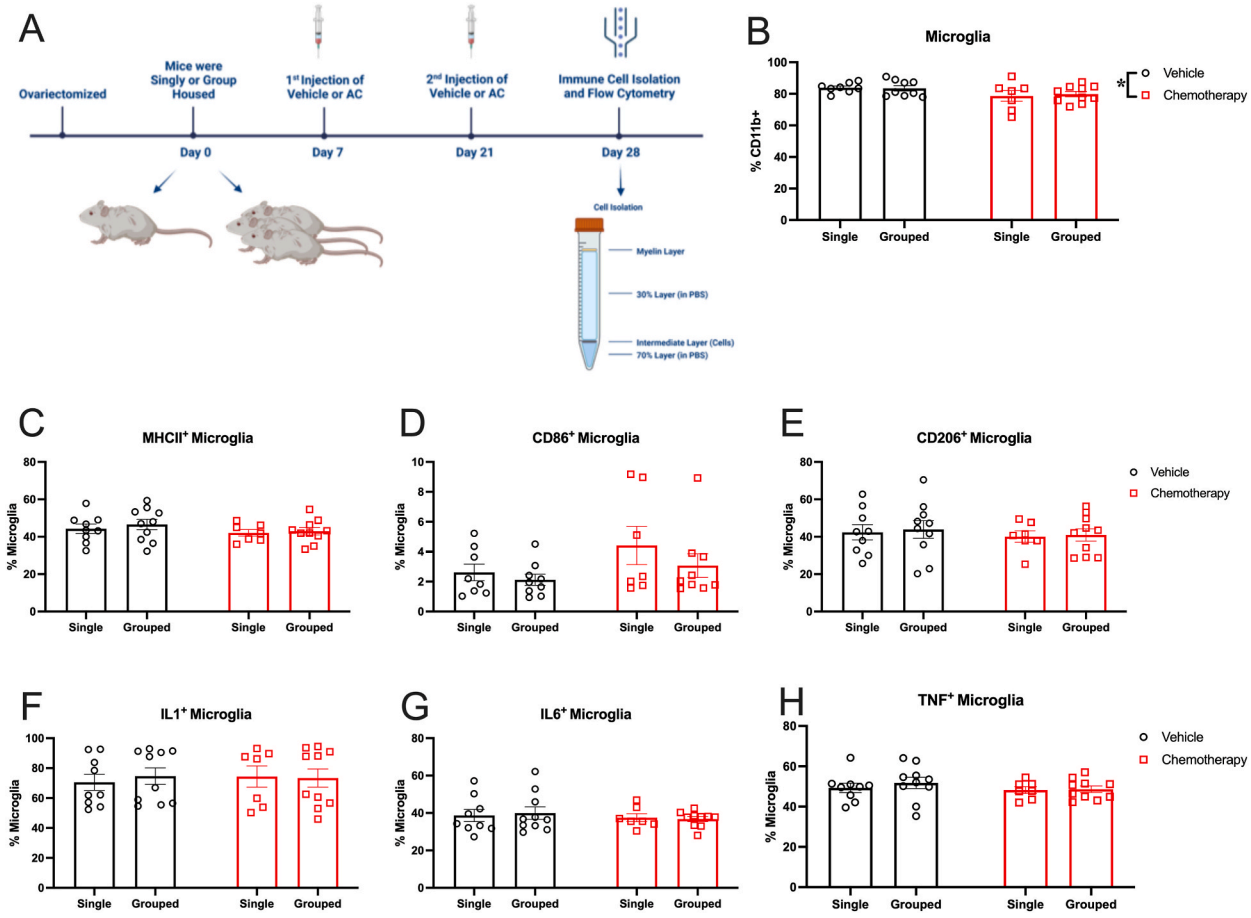

Fig. 1. Chemotherapy Treatment has Limited Effects on Microglia. (A) Timeline of the experimental design. (B) AC therapy reduced the percentage of microglia independent of housing status (i.e., main effect of chemotherapy treatment). There were no significant changes in (C) MHCII$^+$, (D) CD86$^+$, or (E) CD206$^+$ microglia. Additionally, there were no effects in (F) IL-1$^+$, (G) IL-6$^+$, or (H) TNF$^+$ microglia. The data are presented as mean + SEM. (A) Made with Biorender.com. (B–H) Two-way ANOVA with Fisher's LSD. *[]Main effect of chemotherapy treatment. n = 7–10/group.

two-fold increase in cancer-specific mortality [33]. Additionally, social support post-diagnosis is associated with a lower risk of death [35].

Previous work from our lab has demonstrated the beneficial effects of social enrichment via attenuation of chemotherapy-induced interleukin-6 mRNA expression and depressive-like behavior in a tumor-free mouse model [36]. However, the cellular sources of neuroinflammation remained undetermined. Thus, the current study sought to identify the cell type responsible for neuroinflammation following chemotherapy administration and determine the neuroinflammatory effects of chemotherapy administration in tumor-bearing mice. Because one of the most significant risk factors for breast cancer development is aging and no study, to our knowledge, has examined the neuroinflammatory effects of chemotherapy administration in aged mice, we sought to investigate the chemotherapy induced neuroinflammation in aged tumor-free and tumor-bearing mice with or without social enrichment. We hypothesized that chemotherapy increases neuroinflammation via increased immune cell trafficking within the brain and predicted this effect would be modulated by social enrichment.

2. Results

2.1. Neuroinflammatory effects of chemotherapy in aged non-tumor bearing mice

Because aging can prime microglia leading to an exaggerated immune response to an inflammatory stimulus [37] and

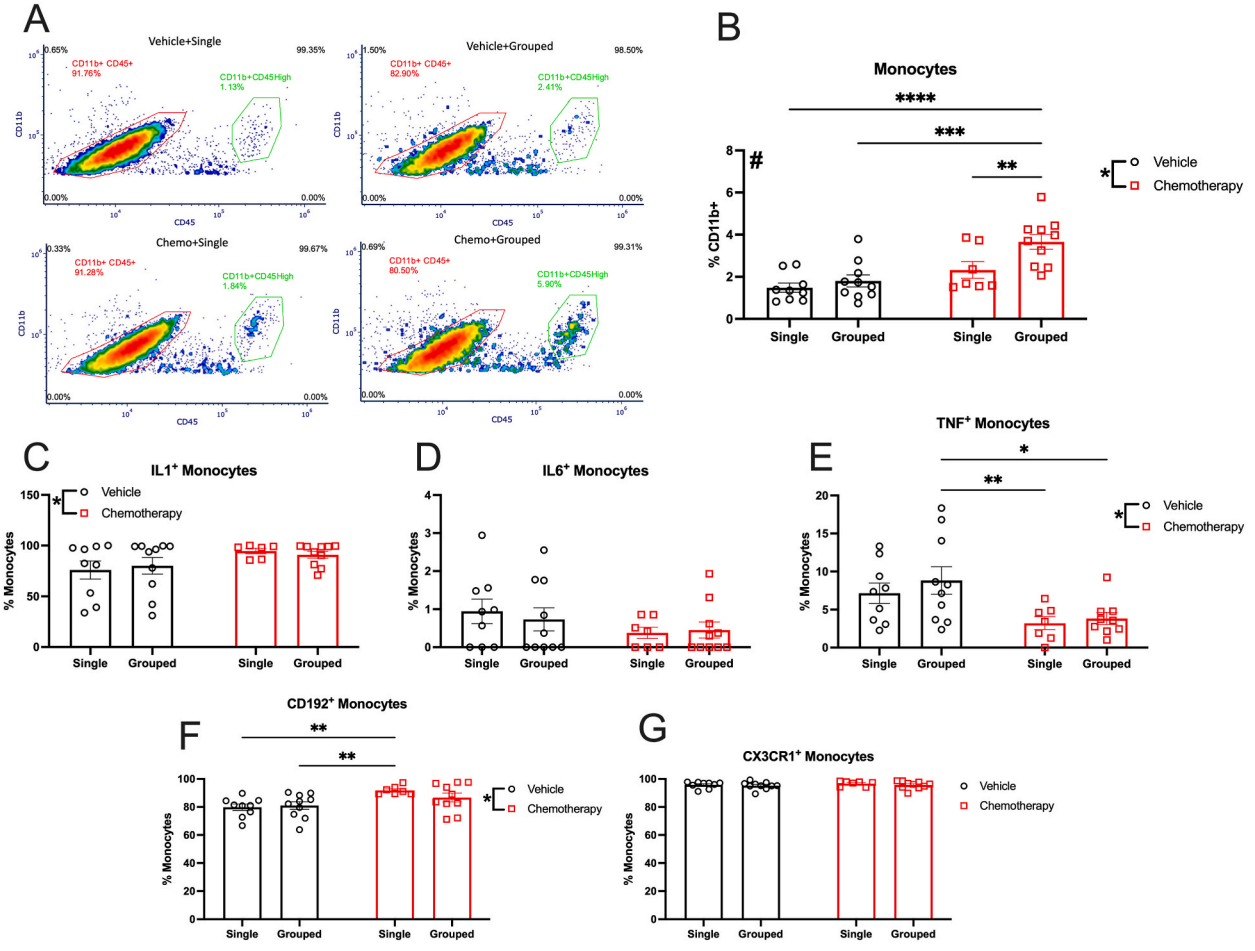

Fig. 2. Chemotherapy Alters Monocyte Trafficking within the Brain. (A) Density plots outlining primarily microglia ($CD11b^{+}$ $CD45^{low}$) and peripheral immune cells ($CD11b^{+}$ $CD45^{high}$). (B) Group housed mice receiving chemotherapy demonstrated a significantly increased percentage of monocytes relative to all other groups. (C) AC therapy increased the percentage of IL-1^{+} monocytes independent of housing status (i.e., main effect of chemotherapy treatment). (D) There were no effects on IL-6^{+} monocytes. (E) Independent of housing status, mice receiving AC therapy displayed a reduced number of TNF^{+} monocytes relative to group housed vehicle treated mice. (F) Singly housed mice receiving chemotherapy exhibited increased percentage of $CD192^{+}$ monocytes relative to vehicle treated groups. (G) There were no effects on $CX3CR1^{+}$ monocytes. The data are presented as mean + SEM. (B–G) Two-way ANOVA with Fisher's LSD. *[Main effect of chemotherapy treatment. #Main effect of housing status. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; Fisher's LSD. n = 6–10/group.

chemotherapy treatment alone in young mice induces a neuroinflammatory response [36], we first sought to determine the effects of chemotherapy treatment in aged mice. Chemotherapy treatment had minor effects on microglia (Fig. 1). Specifically, independent of housing status, mice receiving chemotherapy treatment displayed reduced number of microglia as a percentage of CD11b$^+$ cells (Fig. 1B; $F_{1,30} = 4.954$, $p < 0.05$). However, direct comparisons between groups revealed no significant differences (Fig. 1B). AC therapy had no significant effect on microglial phenotypes (Fig. 1C–H). Specifically, there were no changes in the percentages of MHCII$^+$, CD86$^+$, or CD206$^+$ microglia (Fig. 1C–E). Additionally, chemotherapy treatment did not affect the percentages of microglia expressing pro-inflammatory cytokines IL-1, IL-6, or TNF (Fig. 1F–H) or anti-inflammatory cytokines IL-4 and IL-10 (data not shown).

Next, we assessed the effects of chemotherapy treatment on peripheral immune cell trafficking within the CNS. There were main effects of housing (Fig. 2B; $F_{1,32} = 6.637$, $p < 0.05$) and chemotherapy treatment (Fig. 2B; $F_{1,32} = 17.71$, $p < 0.001$) on the number of monocytes as a percentage of CD11b$^+$ cells within the brain (Fig. 2B). Group housed mice receiving chemotherapy demonstrated a significant increase in the percentage of monocytes relative to all other groups (Fig. 2A–B, $p < 0.01$ for all comparisons). Additionally, independent of housing status, mice receiving AC therapy displayed an increased number of IL-1$^+$ monocytes as a percentage of total monocytes (Fig. 2C; $F_{1,31} = 4.339$, $p < 0.05$). Direct comparisons between groups revealed no significant differences other than the main effect of chemotherapy treatment (Fig. 2C). As a likely consequence, there was a main effect of chemotherapy treatment on the percentage of TNF$^+$ monocytes (Fig. 2E; $F_{1,31} = 10.71$, $p < 0.01$). Specifically, independent of housing status, mice receiving AC therapy had a reduced percentage of TNF$^+$ monocytes relative to group housed vehicle-treated mice (Fig. 2E, $p < 0.05$). There was no effect on IL-6$^+$ monocytes (Fig. 2D). Chemotherapy altered expression of monocyte cell surface markers (Fig. 2F). There was a main effect of chemotherapy treatment on the percentage of CD192$^+$ monocytes (Fig. 2F; $F_{1,32} = 11.16$, $p < 0.01$). Singly housed mice receiving chemotherapy exhibited an increase in CD192$^+$ monocytes relative to both vehicle treated groups (Fig. 2F; $p < 0.05$). However, there was no effect on CX3CR1 expression by monocytes (Fig. 2G).

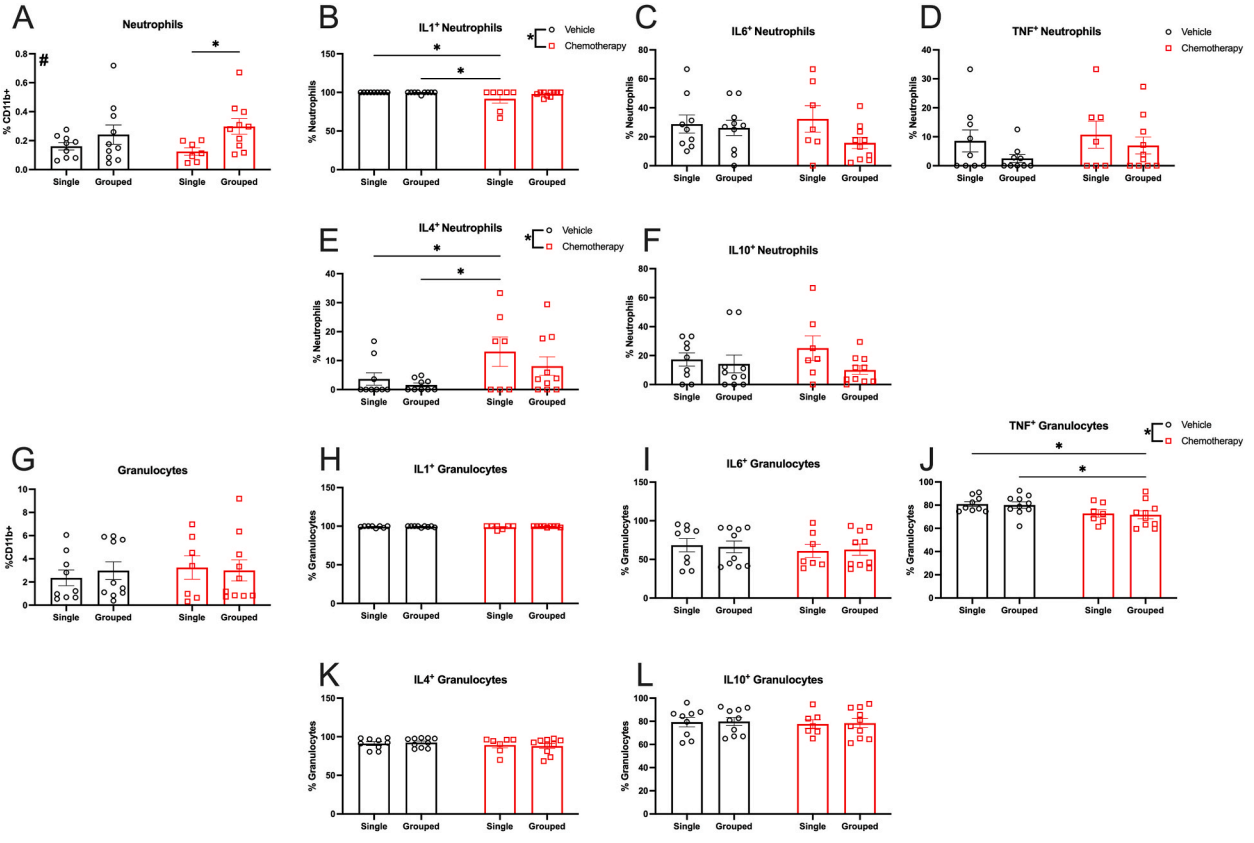

Fig. 3. Effects of Chemotherapy Administration on Neutrophils and Granulocytes within the Brain. (A) AC therapy increased the percentage of neutrophils in group housed mice relative to their singly housed counterparts. (B) Chemotherapy treatment reduced the percentage of IL-1$^+$ neutrophils; singly housed chemotherapy treated mice exhibited reduced IL-1$^+$ neutrophils relative to both vehicle groups. There were no significant effects on (C) IL-6$^+$, (D) TNF$^+$, or (F) IL-10$^+$ neutrophils. (E) AC therapy increased the percentage of IL-4$^+$ neutrophils; singly housed chemotherapy treated mice demonstrated increased IL-4$^+$ neutrophils relative to both vehicle groups. There were no significant effects on the percentage of (G) granulocytes or the percentage of granulocytes expressing (H) IL-1, (I) IL-6, (K) IL-4, or (L) IL-10. (J) AC treatment reduced the number of TNF$^+$ granulocytes; group housed chemotherapy treated mice exhibited reduced TNF$^+$ granulocytes relative to both vehicle groups. The data are presented as mean + SEM. (A–L) Two-way ANOVA with Fisher's LSD. *[Main effect of chemotherapy treatment. #Main effect of housing status. *$p <$ 0.05; Fisher's LSD n = 7–10/group.

Similar to monocytes, there was a main effect of housing on the percentage of neutrophils within the brain (Fig. 3A; $F_{1,32} = 6.198$, $p < 0.05$). Group housed mice receiving AC demonstrated a significantly increased percentage of neutrophils relative to singly housed mice that were administered chemotherapy (Fig. 3A; $p < 0.05$). Additionally, there were main effects of chemotherapy treatment on IL-1^+ (Fig. 3B; $F_{1,31} = 4.901$, $p < 0.05$) and IL-4^+ (Fig. 3E; $F_{1,31} = 7.133$, $p < 0.05$) neutrophils. Singly housed mice receiving chemotherapy exhibit a significant reduction in the percentage of IL-1^+ neutrophils and a significant increase in the percentage of IL-4^+

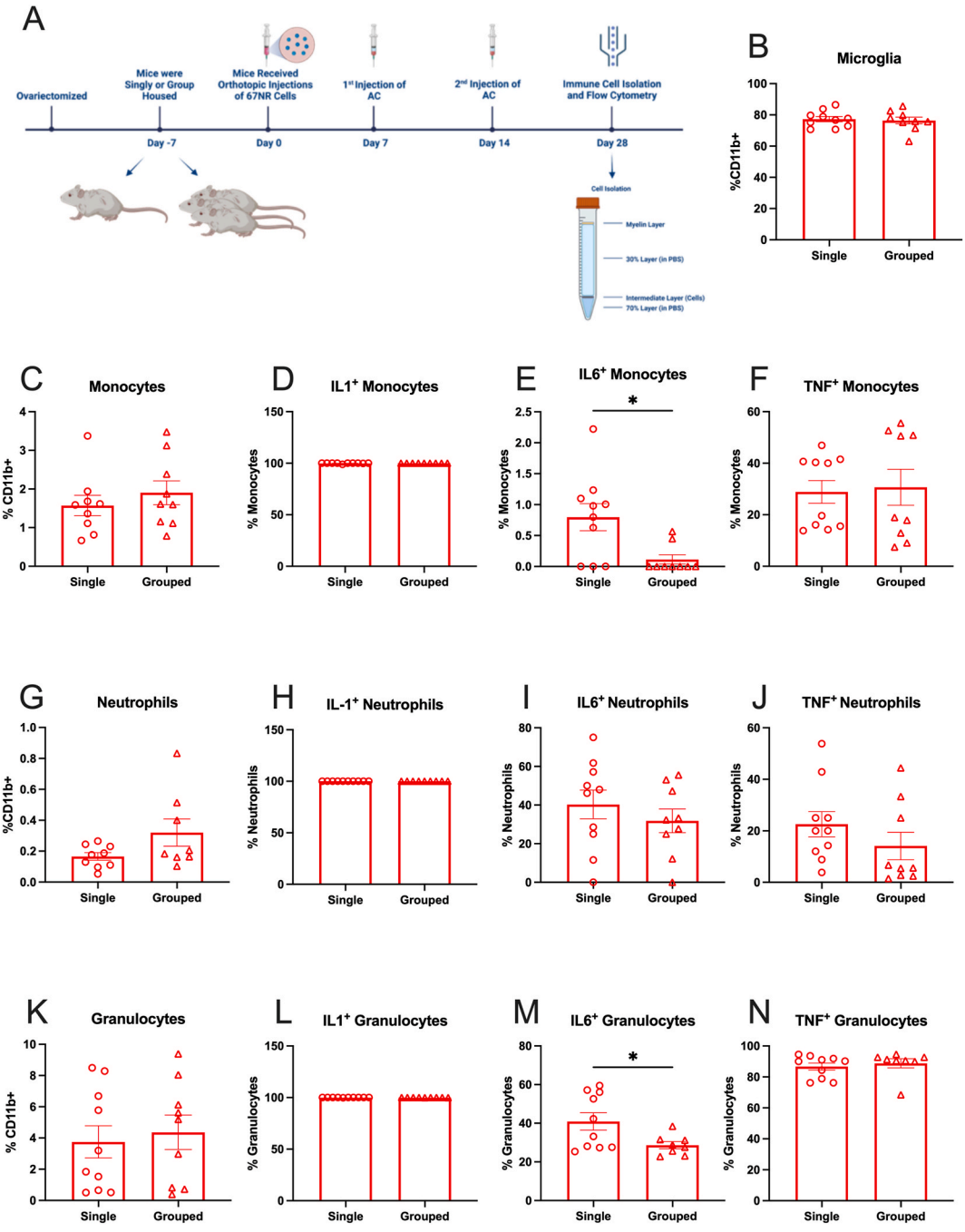

Fig. 4. Social Enrichment Reduces the Percentage of IL-6^+ Monocytes and Granulocytes in Brain of Tumor Bearing Mice Administered Chemotherapy. (A) Timeline of experimental design. (B) Social enrichment did not alter the percentage of microglia within the brain. Group housing tumor bearing mice receiving AC therapy did not affect (C) monocytes, (D) IL-1^+ monocytes, (F) TNF$^+$ monocytes, (G) neutrophils, (H) IL-1^+ neutrophils, (I) IL-6^+ neutrophils, (J) TNF$^+$ neutrophils, (K) granulocytes, (L) IL-1^+ granulocytes, or (N) TNF$^+$ granulocytes. However, social enrichment in tumor bearing mice receiving chemotherapy reduced the percentage of IL-6+ (E) monocytes and (M) granulocytes. The data are presented as mean + SEM. (A) Made with Biorender.com. (B–N) Student's *t*-tests. *$p < 0.05$. n = 8–10/group.

neutrophils relative to both vehicle treated groups (Fig. 3B and E; $p < 0.05$ for all comparisons). There were no effects on IL-6$^+$, TNF$^+$, or IL-10$^+$ neutrophils (Fig. 3C,D, and F). Similarly, there were no effects in the number of granulocytes as a percentage of CD11b$^+$ cells (Fig. 3G). No effects were observed in the percentage of IL-1$^+$, IL-6$^+$, IL-4$^+$, or IL-10$^+$ granulocytes (Fig. H–I and K-L). There was a main effect of chemotherapy treatment on TNF$^+$ granulocytes (Fig. 3J; $F_{1,32} = 7.694$, $p < 0.05$). Specifically, group housed mice receiving AC therapy exhibited a significantly reduced percentage of TNF$^+$ granulocytes relative to both vehicle treated groups (Fig. 3J, $p < 0.05$).

2.2. Neuroinflammatory effects of chemotherapy in aged tumor bearing mice

Due to the limited translation of chemotherapy treatment alone, we next examined the effects of chemotherapy in aged mice bearing non-metastatic mammary tumors with or without social enrichment provided by similar aged stimulus animals. As with

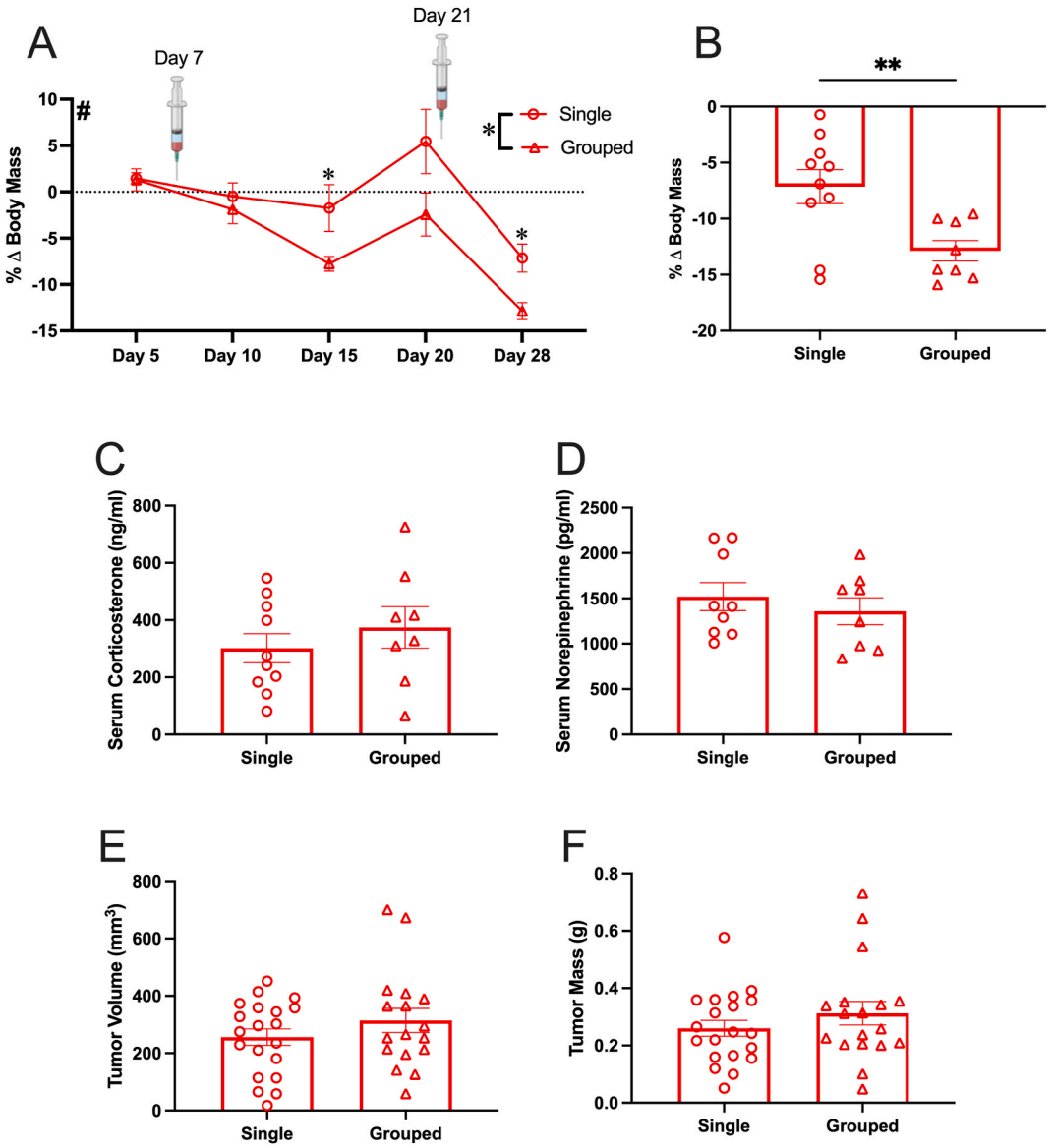

Fig. 5. Social Enrichment with Aged Mice Does Not Alter Stress Measures or Tumor Growth in Tumor Bearing Mice Administered Chemotherapy. (A) Chemotherapy treatment in aged tumor bearing mice results in significant body mass loss. (B) Group housed aged tumor bearing mice demonstrate significantly increased body mass loss relative to their singly housed counterparts. Social enrichment had no effect on serum (C) corticosterone or (D) norepinephrine. Group housing had no effect on (E) tumor volume or (F) tumor mass in aged mice receiving AC therapy. The data are presented as mean + SEM. (A) Mixed-effects analysis with Fisher's LSD. *[I]Main effect of housing status. #Main effect of time. *$p < 0.05$; Fisher's LSD (B–F) Student's *t*-tests. *$p < 0.05$, **$p < 0.01$.(A–D) n = 8–10/group (E–F) n = 17–20/group.

chemotherapy treatment alone, social enrichment had no effect on the percentage of microglia (Fig. 4B), percentage of microglia expressing MHCII, CD86, or CD206 (Suppl. Figs. 1A–C), or percentage of microglia expressing IL-1, IL-6, or TNF (Suppl. Figs. 1D–F) in tumor bearing mice receiving AC therapy. Social enrichment had no effects on the percentages of monocytes (Fig. 4C), neutrophils (Fig. 4G), and granulocytes (Fig. 4K). Additionally, social enrichment did not alter the percentage of these cells expressing IL-1 (Fig. 4D,H, and L) or TNF (Fig. 4F, J, and N). However, social enrichment reduced the percentages of IL-6^{+} monocytes (Fig. 4E; $t = 2.823$, $p < 0.05$) and granulocytes (Fig. 4M; $t = 2.322$, $p < 0.05$) within the brain, suggesting a potential anti-inflammatory effect.

When examining the effects on body mass of AC therapy in aged tumor bearing mice with or without social enrichment, we

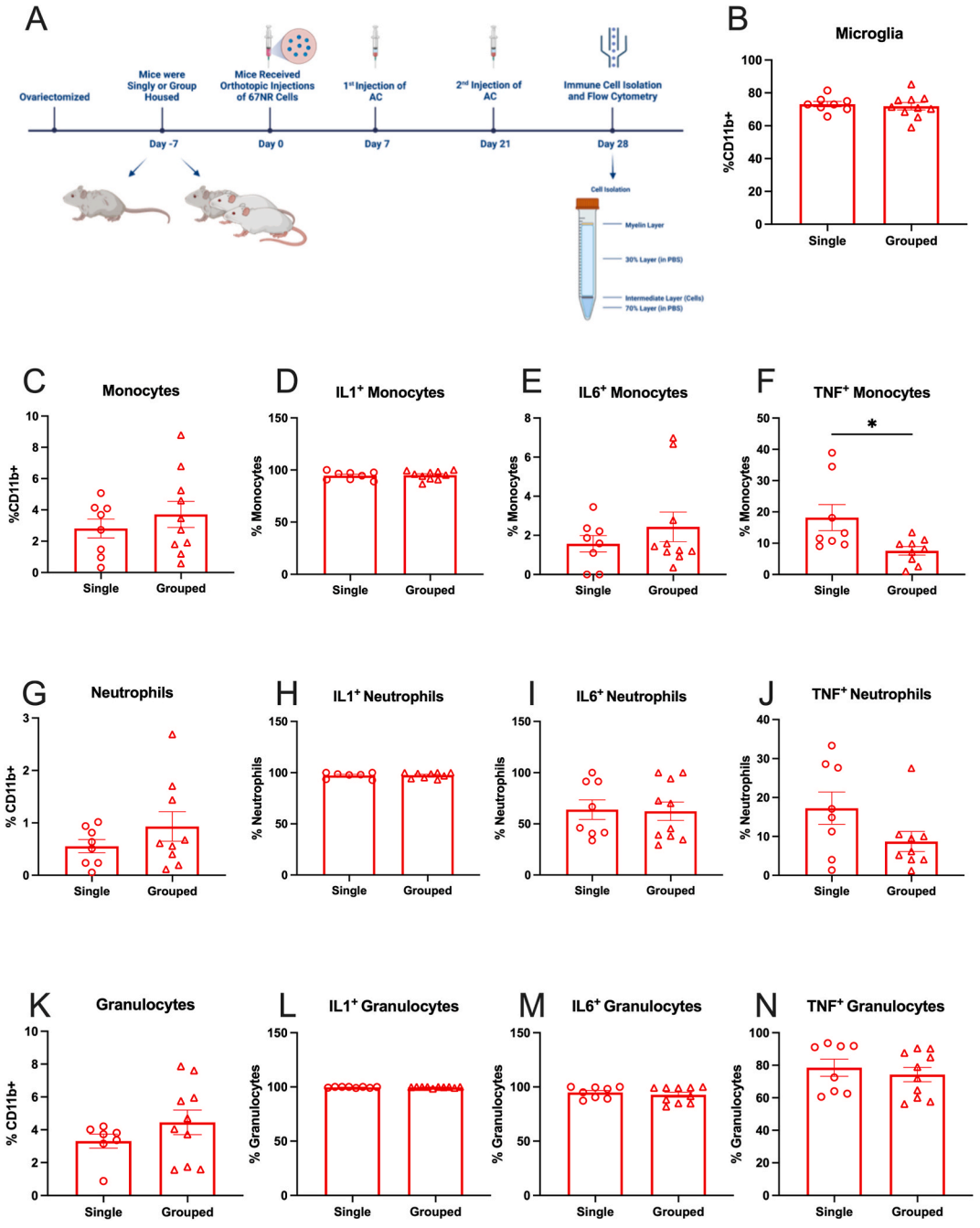

Fig. 6. Social Enrichment with Young Adult Mice Reduces TNF + Monocytes within the Brain of Aged Tumor Bearing Mice Receiving AC Therapy. (A) Timeline of experimental design. Social enrichment with young mice had no effect on (B) microglia, limited effects on (C–F) monocytes, and no effect on (G–J) neutrophils or (K–N) granulocytes. The data are presented as mean + SEM. (A) Made with Biorender.com. (B–N) Student's *t*-tests. *$p < 0.05$. $n = 7$–10/group.

observed a main effect of housing (Fig. 5A; $F_{1,17} = 5.367$, $p < 0.05$) and time (Fig. 5A; $F_{2.014,32.22} = 19.55$, $p < 0.001$). Specifically, group housed tumorbearing mice receiving AC therapy demonstrated significantly increased body mass loss relative to their singly housed counterparts (Fig. 5B; $t = 3.034$, $p < 0.01$). However, there were no significant effects of social enrichment on measures of stress; group housing did not alter serum corticosterone (Fig. 5C) or serum norepinephrine (Fig. 5D) concentrations. Additionally, social enrichment had no effect on tumor growth in aged mice receiving AC therapy; tumor volume (Fig. 5E) and tumor mass (Fig. 5F) were similar between groups.

2.3. *Effects of chemotherapy treatment in aged tumor bearing mice with or without young adult mice social enrichment*

Because social enrichment has previously been demonstrated to be beneficial in suppressing tumor growth in young adult mice [38] and these effects were not observed in the previous cohort when examining aged mice, we sought to determine whether the age of the group housed partners may play a role. Specifically, we aimed to elucidate whether social enrichment with young adult mice could reduce the neuroinflammatory effects of chemotherapy and alter tumor growth in aged tumor bearing mice. Social enrichment with

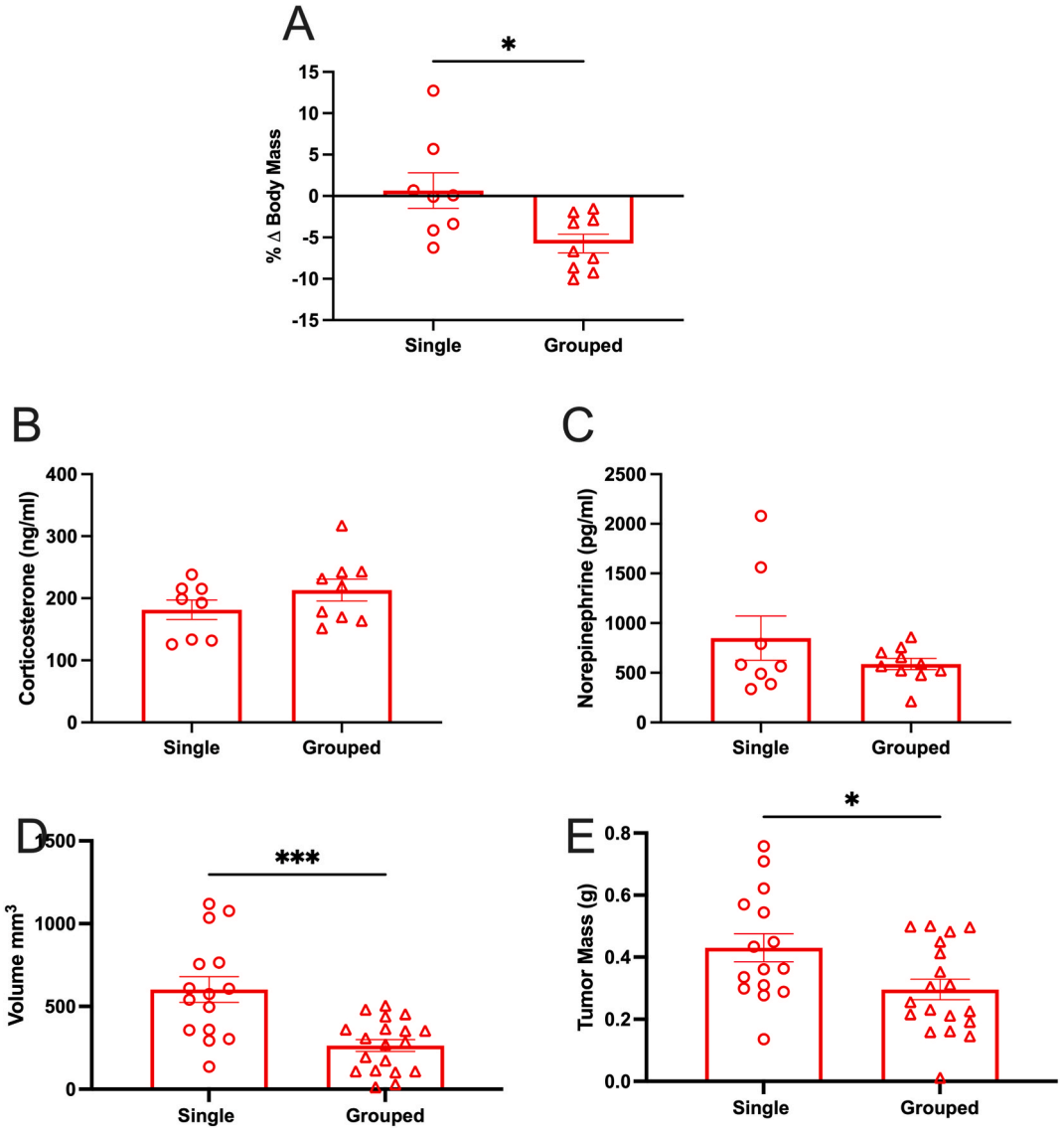

Fig. 7. Social Enrichment with Young Adult Mice Reduces Tumor Volume and Mass in Aged Tumor Bearing Mice Receiving AC Therapy. (A) Aged tumor bearing mice group housed with young mice demonstrate significantly increased body mass loss relative to their singly housed counterparts. There were no effects of social enrichment with young mice on serum (B) corticosterone or (C) norepinephrine. Social enrichment with young mice significantly reduced (D) tumor volume and (E) tumor mass. (A–E) Student's *t*-tests. *$p < 0.05$, ***$p < 0.001$. (A–C) $n = 8$–10/group (E–F) $n = 15$–19/group.

young adult mice had limited effects on immune cell composition within the brain relative to singly housed aged tumor bearing mice receiving chemotherapy. Social enrichment with young adult stimulus mice did not affect the percentage of microglia (Fig. 6B) or microglia phenotypes (Suppl. Figs. 2A–F). Additionally, there were no effects on the percentages of neutrophils (Fig. 6G–J) or granulocytes (Fig. 6K-N). However, there were limited effects on monocytes within the brain (Fig. 6C–F); social enrichment with young adult mice significantly reduced the percentage of TNF^+ monocytes relative to aged singly housed tumor bearing mice receiving AC therapy (Fig. 6F). Similar to social enrichment with aged mice, group housing tumor bearing mice receiving AC therapy with young mice significantly increased body mass loss relative to their singly housed counterparts (Fig. 7A). Additionally, there were no effects on serum corticosterone (Fig. 7B) or serum norepinephrine (Fig. 7C) concentrations. However, contrary to group housing with aged mice, social enrichment with young mice significantly reduced tumor volume (Fig. 7D; $t = 4.242$, $p < 0.001$) and tumor mass (Fig. 7E; $t = 2.445$, $p < 0.05$) in aged mice receiving AC therapy relative to their singly housed counterparts. These data demonstrate that the age of social enrichment partners (i.e. the stimulus mice) can have a significant effect on tumor growth.

3. Discussion

Aging is a primary risk factor for the development of breast cancer. Breast cancer patients frequently report alterations in behavior and cognition, which typically worsen with chemotherapy treatment [1–4,12]. Foundational science studies have supported the associations among breast cancer, chemotherapy, and alterations in behavior and cognition [5–8]. However, to our knowledge, all foundational studies, to date, have examined these associations in young adult rodents. Thus, the current study sought to examine these neuroinflammatory effects of chemotherapy in aged tumor naïve and tumor bearing mice. Additionally, due to the previous reported effects of social enrichment on attenuating neuroinflammation and reducing tumor growth [36,38], we sought to determine whether social enrichment may produce similar effects in aged mice.

First, we examined the neuroinflammatory effects of chemotherapy treatment in aged tumor naïve mice with or without social enrichment with aged partners. Similar to our previous studies in young adult mice [36], chemotherapy treatment in aged tumor naïve mice had a minimal effect on microglia (Fig. 1). AC therapy did not shift microglia to a pro-inflammatory ($MHCII^+$ or $CD86^+$; Fig. 1C–D) or anti-inflammatory phenotype ($CD206^+$; Fig. 1E) nor did chemotherapy treatment alter the secretion of pro-inflammatory (IL-1, IL-6, TNF; Fig. 1F–H) or anti-inflammatory cytokines (IL-4 and IL-10; data not shown). Chemotherapy treatment reduced the number of microglia as a percentage of $CD11b^+$ cells (Fig. 1B). However, this is likely due to increased peripheral immune cell trafficking, as opposed to a reduction in the number of microglia (Figs. 2 and 3). These data replicate our findings in young adult mice treated with AC therapy [36]. However, these findings should not be overgeneralized to all chemotherapeutics, as previous studies using methotrexate and paclitaxel have observed microglial activation following chemotherapy administration [39–41]. Indeed, administration of paclitaxel (30 mg/kg, i. p.) injected in 6 doses every other day for 11 days did increase microglia secretion of IL-1, but not IL-6 or TNF [41]. Notably, this increase was only observed 6 h after the last injection and had subsided by 24 h, suggesting a short-term effect of microglia activation. Treatment with methotrexate (100 mg/kg i. p. weekly/3weeks) resulted in an activation of microglia both in vivo and in vitro, with the in vivo effects lasting up to six months [39]. Future studies should assess the effects of chemotherapeutics on microglia across multiple timepoints using alternate methods to the more traditional methods of immunohistochemistry, ELISAs, and flow cytometry. It is possible that the current method may not be sufficiently sensitive to detect subtle microglial activation; thus, alternative approaches (i.e., RNAseq) may be required to more confidently rule out microglial activation.

The majority of chemotherapeutic effects on immune cells within the brain of aged tumor naïve mice were observed in peripheral immune cells (Fig. 2A). Treatment of aged group housed tumor naïve mice with AC resulted in a significant increase in immune cell trafficking within the brain (Fig. 2A). Specifically, social enrichment in aged tumor naïve mice increased the percentage of monocytes within the brain relative to all other groups (Fig. 2B). Notably, these monocytes are primarily producing IL-1, with approximately 90 % of monocytes being $IL\text{-}1^+$ in socially enriched mice (Fig. 2C). Although there is a lower percentage of monocytes within the brain of singly housed chemotherapy treated mice, there is also a shift towards the majority producing IL-1, approximately 95% (Fig. 2C). Previous studies have demonstrated increased IL-1 secretion within the brain following paclitaxel administration and have attributed these effects to likely microglia activation [41]. Indeed, increased IL-1 within the hippocampus was observed 24 h following the last injection. However, the authors had previously demonstrated the effects on microglia are short-term (i.e., lasting ~6 h). This suggests that other cells likely contribute to the increased IL-1, and based on the current data, monocytes would be an ideal candidate. Mice receiving chemotherapy treatment increased their expression of CD192, a key mediator of monocyte migration (Fig. 2F) [42]. Specifically, singly housed mice administered AC had an increased percentage of $CD192^+$ monocytes relative to both vehicle treated groups (Fig. 2F). In addition, to being a key regulator of monocyte migration, CD192 is also a marker of classical monocytes (i.e., inflammatory monocytes) [43]. Thus, an increase in CD192 is not unexpected because of the significant increase in monocyte trafficking in group housed mice and the vast majority of monocytes producing IL-1 independent of housing status. Interestingly, independent of housing status, AC therapy reduced the number of monocytes producing TNF (Fig. 2E). However, the significance of reduced TNF production by monocytes remains to be determined. Chemotherapy treatment in aged tumor naïve mice had modest effects on neutrophils (Fig. 3) As observed with monocytes, social enrichment in aged tumor naïve mice increased the percentage of neutrophils within the brain relative to singly housed chemotherapy treated mice (Fig. 3A). Additionally, independent of housing, there were main effects of chemotherapy treatment reducing IL-1 expression and increasing IL-4 expression within neutrophils (Fig. 3B and E). This increased expression of IL-4 by neutrophils likely reflects a shift toward resolution of inflammation [44]. From these data, there are questions that remain and should be the subject for future investigations. First, contrary to our hypotheses, social enrichment did not attenuate peripheral immune cell trafficking in aged mice receiving AC therapy. Indeed, social enrichment enhanced monocyte and neutrophil trafficking; the functional significance and effects on behavior remained to be determined. Second, the source of

recruitment of peripheral immune cells within the brain remains unspecified. Based on previous studies, a potential mechanism could be activated microglia, as studies have reported transient microglial activation following chemotherapy administration. The timing of tissue collection following the second injection of chemotherapy (i.e., seven days) could account for the absence of microglial activation in the current study.

Due to the limited translation of chemotherapy treatment alone, we next sought to examine the neuroinflammatory effects of AC therapy in tumor bearing mice with or without social enrichment. Similar to the previous cohort and our previous experiments, social enrichment in aged tumor bearing mice receiving AC therapy had no effect on the percentage of microglia within the brain, microglial phenotypes, or cytokine production by microglia (Fig. 4B and Suppl. Fig. 1). Additionally, social enrichment did not alter the percentages of monocytes (Fig. 4C), neutrophils (Fig. 4G), or granulocytes (Fig. 4K) within the brain. However, within monocytes and granulocytes, group housing did alter cytokine production. Specifically, the percentages of IL-6^{+} monocytes (Fig. 4E) and IL-6^{+} granulocytes (Fig. 4F), were reduced by social enrichment in aged tumor bearing mice receiving AC therapy; this suggests that social enrichment is effective in diminishing the neuroinflammatory effects in aged mice. We have observed in previous experiments that socially isolated mice receiving AC therapy displayed increased depressive-like behavior with a concurrent increase in hippocampal IL-6 expression, whereas, group housing attenuated AC-induced IL-6 and depressive-like behavior [36]. Chemotherapy administration to patients increases serum IL-6 concentrations [45–47]. Increased IL-6 signaling within the serum of patients receiving chemotherapy is frequently correlated with increased cognitive decline and alterations in behavior [45,48,49]. Additionally, our lab has previously demonstrated that a single dose of AC therapy increases NREM sleep, REM sleep, and sleep fragmentation. All of which were positively correlated with IL-6 expression within the hippocampus [17]. Thus, although not explicitly tested within the current study, these data suggest that social enrichment in aged tumor bearing mice may be beneficial in attenuating chemotherapy induced behavioral deficits. Future studies should examine the effects of social enrichment in aged tumor bearing mice on affective behavior, cognitive function, and sleep.

Social enrichment by aged stimulus animals did not alter tumor volume (Fig. 5E) or tumor mass (Fig. 5F) among aged mice. This is contrary to previous experiments demonstrating that social isolation increases tumor growth [38,50,51]. Most studies propose that the alterations in tumor growth are a consequence of an increased stress response. Thus, no changes in tumor growth was consistent with the lack of differences in measures of stress between singly and socially enriched groups (Fig. 5C and D). However, social enrichment in young rodents increases circulating oxytocin concentrations [52] and oxytocin has both immunomodulatory properties and potential oncostatic effects [53,54,]. Thus, the question remains why no effect was observed in tumor growth. Recent studies in aged mice demonstrate that social enrichment with aged partners does not result in increased oxytocin secretion. Instead, social enrichment with young partners resulted in the expected increase in oxytocin section in aged mice [55]. Thus, we next examined the neuroinflammatory effects of AC therapy in aged tumor bearing mice with or without social enrichment with young adult partners. Similar to previous cohorts, social enrichment with young partners significantly reduced neuroinflammation in tumor bearing mice relative to their singly housed counterparts. Specifically, social enrichment with young partners reduced the percentage of TNF^{+} monocytes within the brain (Fig. 6F). Most notably, social enrichment with young partners significantly reduced tumor volume and tumor mass at the time of tissue collection (Fig. 7D–E), suggesting that the age of social enrichment partners plays a role in the effect of social enrichment on tumor growth. This effect is likely mediated via increased circulating oxytocin concentrations and not a consequence of altered stress response as measures of stress were not different between groups (Fig. 7B–C). Future studies in aged mice are needed to further elucidate whether these effects are due to increased circulating oxytocin concentrations and determine what effect social enrichment in aged mice with young partners is having on the tumor microenvironment.

The current study examined the neuroinflammatory effects of chemotherapy in aged, tumornaïve and tumorbearing mice with or without social enrichment using one non-metastatic cell line for tumor induction and one chemotherapeutic treatment. Future studies should expand to include additional chemotherapeutics and tumor models. Additionally, studies should expand to include combination treatments such as anti-hormonal therapies, radiation therapies, and anti-angiogenic therapies that are often paired with chemotherapy in the treatment of breast cancer. Furthermore, the current study assessed neuroinflammation at one time point, seven days following the second chemotherapy injection. Although the timing of tissue collection was based on previously published data demonstrating time-specific neuroinflammation, future studies should assess the effects of chemotherapeutics on microglia across multiple timepoints and use converging methods, such as single-cell RNA sequencing, to determine subtle changes in microglia gene expression. Mice underwent an ovariectomy procedure to eliminate the confound of acute ovarian failure in response to chemotherapy administration. There is the potential for post-surgical effects of ovariectomy that was not examined in the current study. Thus, subsequent studies should determine the potential post-surgical effects of an ovariectomy procedure. Most notably, future studies are needed to determine the causal mechanism by which social enrichment in aged mice with young partners reduces tumor growth.

4. Conclusions

Aging is a primary risk factor for the development of breast cancer. Breast cancer patients frequently report alterations in behavior and cognition. Foundational science studies have supported the associations among neuroinflammation, breast cancer, chemotherapy, and alterations in behavior and cognition. However, to date, no studies have examined these associations in young adult rodents. The current study is the first to examine the neuroinflammatory effects of chemotherapy in aged tumor naïve and tumor bearing mice with or without social enrichment. Social enrichment enhanced peripheral immune cell trafficking in aged tumor naive mice treated with AC. However, social enrichment in aged tumor bearing mice receiving AC reduced the percentage of IL-6^{+} monocytes and granulocytes relative to their singly housed counterparts, demonstrating the ability of social enrichment to attenuate some aspects of neuroinflammation in aged mice. There was no effect on tumor growth when social enrichment partners were also aged. Social enrichment

with young mice, however, reduced neuroinflammation, tumor volume, and tumor mass in aged tumor bearing mice relative to their singly housed counterparts. These data add to the literature detailing the beneficial effects of social enrichment and are the first to demonstrate that social support with young housing partners reduces tumor growth in aged mice.

5. Methods

5.1. Mice

All experiments were approved and conducted in accordance with guidelines set by the West Virginia University Institutional Animal Care and Use Committee. Adult Balb/C female mice were acquired from Charles River Laboratories and group housed (5 mice/cage) in a 14:10 light-dark cycle. Mice received *ad libitum* access to reverse osmosis water and food (Envigo Teklad #2018). Mice were continuously group housed and aged in house until ~20 months of age. After aging, mice underwent an ovariectomy procedure to eliminate the confound of acute ovarian failure in response to chemotherapy administration [56,57]. Specifically, mice were anesthetized via 2% isoflurane and placed prone. A small midline incision (~1 cm) was made to allow for movement and visualization of each ovarian fat pad. A 0.5 cm incision was made directly over each ovarian fat pad and the ovaries and uterine horn were withdrawn for visualization. The ovaries were removed from the uterine horn via cauterization. The uterine horn was returned into the abdominal cavity and the incision was closed using tissue glue. Mice were allowed one week recovery prior to any additional experimental manipulation.

5.2. Neuroinflammatory effects of chemotherapy in aged non-tumor bearing mice

One week following the ovariectomy procedure, mice were randomly assigned groups (i.e., single or group housing and vehicle or chemotherapy; n = 10/group). Group housing consisted of triads with one experimental mouse and two age matched, familiar, ovariectomized female Balb/C mice. One week after housing mice received their first intravenous (IV; tail vein) injection of vehicle (0.9% saline) or chemotherapeutic cocktail (9 mg/kg doxorubicin (A), NDC# 0069-3031-20; 90 mg/kg cyclophosphamide (C), NDC# 70121-1238-1). Two weeks later, mice received their second injection of vehicle or AC. Seven days following their second injection, mice received an intraperitoneal injection of Euthasol (NDC# 051311-050-01) to allow for sedation. After verifying sedation via a toe pinch, mice were transcardially perfused with ice cold 1X phosphate-buffered saline (Gibco, Cat# 10010-023) for ~2 min and decapitated. Brains were extracted and immune cells were isolated and processed as described below. Tissue collection occurred at ~ ZT4 (i.e., 4 h after lights on). The timeline for injections, tissue collection, and dose of chemotherapeutic cocktail were based on previous time-course data within our lab [36]. The dose of chemotherapeutic cocktail 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. The human equivalent dose was calculated using body surface area [58]. Of note, this experiment was repeated four times with four independent cohorts of mice (n = 10/cohort). During the experiment, three mice were euthanized prior to completion of the study (n = 2 single + chemotherapy and n = 1 single + vehicle). Additionally, one mouse was excluded prior to analysis due to a technical failure in immune cell extractions (n = 1 single + chemotherapy).

5.3. Neuroinflammatory effects of chemotherapy in aged tumor bearing mice

Following recovery from ovariectomy procedure, mice were randomly assigned to single or group housing (triads) (n = 10/group). Group housing was performed as described previously (i.e., two aged matched, familiar, ovariectomized female Balb/C mice). One week following single or group housing, mice received 100 μl bilateral orthotopic injections of the non-metastatic mouse mammary cancer cell line 67NR (1.0×10^5 cells/injection; Barbara Ann Karmanos Cancer Institute, Detroit, MI) into the 4th and 9th inguinal mammary glands. Body mass and tumor measurements were obtained every five days. Tumor volume was calculated using the following formula: tumor volume = (length × width ^{2})/2 [59]. One week after tumor implantation, mice received their first IV injection of chemotherapeutic cocktail (9 mg/kg doxorubicin (A) and 90 mg/kg cyclophosphamide (C)). Two weeks later mice received their second IV injection of AC therapy. Seven days following their second injection, a submandibular blood sample was obtained to measure corticosterone and norepinephrine concentrations within the serum. Immediately following the blood sample, mice were transcardially perfused as previously described. Brains were extracted and immune cells were isolated as described below. Tissue collection occurred at ~ ZT4. This experiment was repeated twice with two independent cohorts (n = 10/cohort). Due to the limitation on the number of aged mice, all mice received 67NR injections and AC therapy (i.e., there were no vehicle aged mice). One mouse died prior to tumor injection (n = 1 grouped).

5.4. Neuroinflammatory effects of chemotherapy in aged tumor bearing mice with or without young adult mice social enrichment

After ovariectomy, mice were randomly assigned to single or group housing (n = 10/group). In contrast to the previous experiments, group housing consisted of triads with one experimental aged mouse (>20 months) and two young adult (~8–9 weeks old), novel, ovariectomized female Balb/C mice. The young adult mice were obtained from Charles Rivers Laboratories and allowed one week to acclimate prior to any experimental manipulation. One week after arrival, the young adult mice underwent an ovariectomy as previously described. Young adult mice were allowed one week recovery prior to group housing. One week following housing all experimental mice (i.e., aged mice) received 67NR cell injections as previously described. Tumor measurements were obtained every

five days. Seven days after 67NR cell injections, mice received their first IV injection of AC therapy and two weeks later their second IV injection of AC therapy. Seven days following their second injection a submandibular blood sample was obtained and mice were euthanized as previously described. Tissue collection occurred at ~ ZT4. This experiment was repeated twice with two independent cohorts (n = 10/cohort). Due to the limitation on the number of aged mice, all mice received 67NR injections and AC therapy. Two mice died prior to completion of the experiment (n = 2 single housed).

5.5. 67NR cell line

67NR cells were obtained from Barbara Ann Karmanos Cancer Institute (Detroit, MI). 67NR cells are a non-metastatic murine mammary cancer cell line that was isolated from a single spontaneous Balb/cfC3H mammary tumor [60]. 67NR cells were cultured using sterile technique in DMEM + GlutaMAX (Gibco, Cat# 10566-016) supplemented with 10 % heat-inactivated fetal bovine serum (Cytiva, Cat# SH30070.03), 1 % antibiotic-antimycotic (Gibco, Cat# 15240-062), and 0.1 % MEM Non-Essential Amino Acids (Gibco, Cat# 11140-050). Cells were incubated at 37 °C in a mixture of 5 % carbon dioxide and 95 % air. Once cells reached ~90 % confluence, cell numbers were determined with a 1:1 mixture of trypan blue using an automated hemocytometer (Countess II, Life Technologies). Cells were diluted in DMEM to make a final concentration of 1×10^5 cells per 100 μl in preparation for injection.

5.6. Corticosterone and norepinephrine ELISA

Blood samples were centrifuged at 2500 g for 25 min. The serum was removed and placed in a new tube and stored at −80 °C until ELISA initiation. Serum corticosterone and norepinephrine concentrations were determined using commercially available kits. Specifically, serum corticosterone concentrations were determined via corticosterone ELISA according to the manufacturer's instructions (Arbor Assays, Cat# K014–H1W). Additionally, serum norepinephrine concentrations were measured via a norepinephrine ELISA kit according to the manufacturer's instructions (Abcam, Cat# ab287789). ELISAs were run simultaneously to prevent serum freeze-thaw and both ELISAs were read using a SpectraMax iD3 plate reader (Molecular Devices).

5.7. Immune cell isolation and flow cytometry

Immune cell isolation was adapted from a previously described protocol [61]. Briefly, once brains were extracted they were placed in 10 mL Potter-Elvehjem Tissue Grinder with PTFE Pestle (Pyrex) containing 3 ml of 0.2 % Glucose (Fisher Scientific, Cat# D16-3) in DPBS (Gibco, Cat #14190-136) on ice. Brains were mechanically dissociated by mashing 20–25 times and avoiding bubbles. Once mashed, the pestle was washed with 1 ml of glucose buffer and collected within the homogenizer. All the liquid within the homogenizer was then pipetted through a 40 μm filter (Corning, Cat # 352350) and collected in a 50 ml conical tube. Next, the 50 ml conical tube was spun at 1000 g for 10 min at room temperature. The supernatant was removed and discarded. The cell pellet was resuspended in 3 ml of 70 % Percoll (GE Healthcare, Cat # 17-0891-01) and placed in a clean 15 ml conical tube. 10 ml of 30 % Percoll were slowly layered on top of the 3 ml of 70 %. After layering, the 15 ml tubes were centrifuged at 1200 g for 30 min at room temperature with no break and no acceleration. Following the centrifugation, myelin debris on top was removed with a 5 ml pipette. Immune cells were then isolated from the 30/70 interface using a 5 ml pipette (~2.5 ml) and placed in a clean 15 ml conical tube. Cells were washed by the addition of 5 ml of glucose buffer and inversion of the tube several times. The 15 ml tube was centrifuged at room temperature for 10 min. The supernatant was removed and discarded. The cell pellet was resuspended in 200 μl of PBSAz and transferred to a 96 well U-bottom plate. Cells were pelleted by centrifuging at 1200 rpm for 5 min at 4 °C. The plate was dumped to remove the liquid and cells were washed again in 200 μl of PBSAz. Following pelleting of the cells, 40 μl of Mouse Ig (10 μg/ml; Jackson ImmunoResearch, Cat# 012-000-003) was added and cells were allowed to incubate on ice for 30 min. Following incubation, 100 μl of PBSAz was added and the cells were pelleted via centrifugation. The plate was dumped to remove the liquid and cells were incubated with 20 μl of fluorochrome labeled antibodies (0.4 μg Brilliant Violet 510 anti- CD11b, Biolegend Cat# 101263; 0.125 μg APC-eFluor anti-CD45, Thermo Fisher Scientific Cat# 47–0451082; 0.25 μg APC anti- Ly6C, Biolegend Cat # 128016; 0.5 μg PE-Cy5 anti-Ly6G, Thermo Fisher Scientific Cat #15-9668-82; 0.5 μg Brilliant Blue 700 anti-CD192, BD Biosciences Cat# 747965; 0.25 μg PE-Cy7 anti-CD206, Biolegend Cat # 141720; 0.5 μg APC-R700 anti-CD86, BD Biosciences Cat# 565479; 0.25 μg Brilliant Violet 711 anti-MHCII, Biolegend Cat# 107643; 0.06 μg Brilliant Violet anti-CX3CR1) on ice for 30 min in the dark. After the incubation, 100 μl of PBSAz was added and the cells were pelleted via centrifugation. Cells were then washed 2x with 200 μl of PBSAz and resuspended in 100 μl cytofix/cyoperm buffer (BD Biosciences, Cat# 554714). Cells were incubated at room temperature for 30 min. Cell were pelleted via centrifugation and washed twice with 100 μl of permwash buffer. Next, the cells were resuspended in 0.4 % paraformaldehyde for 20 min, washed twice in 100 μl of brilliant stain buffer (BD Bioscience, Cat # 563794), and stored in brilliant stain buffer overnight. The next morning, the cells were pelleted and resuspended in 100 μl of prewash for 15 min. Following pelleting of the cells, 20 μl of fluorochrome labeled antibodies (0.25 μg FITC anti-IL1 beta, Biorbyt Cat# orb15831; 0.25 μg PE anti-IL-6, Biolegend 504504; 0.125 μg Brilliant Violet 785 anti-TNF alpha, Biolegend Cat # 506341; 0.4 μg Brilliant Violet 605 anti-IL4, Biolegend Cat# 504126; PE-Dazzle 594 anti-IL10, Biolegend Cat# 505034) were added for 30 min at room temperature. 100 μl of permwash was added and the cells were pelleted via centrifugation. Cells were then washed 2x with 100 μl of permwash and resuspended in 200 μl PBSAz for flow cytometric analysis. Flow cytometry was performed using a 3 laser Cytek Aurora system and SpectroFlo software. No stain controls consisted of unstained isolated brain immune cells. Single stain controls consisted of UltraComp eBeads Plus prepared according to the manufacturer's instructions (Thermo Fisher Scientific, Cat # 01-3333-42). Flow data analysis was performed using FCS Express 7 software. Briefly, the gating strategy was as follows; first, the cell population was identified by SSC-A vs FSC-A. Next, single cells were identified via FSC-A vs

FSC-H. Next, $CD11b^+$ cells were identified and graphed vs CD45 expression. This allowed for identification of $CD11b^+CD45^{low}$ cells (microglia) and $CD11b^+$ $CD45^{high}$ cells (monocytes, neutrophils, and granulocytes). Cell populations were defined as follows: Microglia $CD11b^+$ $CD45^{low}CX3CR1^+$, Monocytes $CD11b^+$ $CD45^{high}$ $Ly6C^+$ $Ly6G^-$, Neutrophils $CD11b^+$ $CD45^{high}$ $Ly6C^+Ly6G^+$, and Granulocytes $CD11b^+$ $CD45^{high}$ $Ly6C^-Ly6G^+$.

5.8. Statistical analysis

Prior to any statistical analysis, outliers were identified and removed using the Grubbs' test. Student's *t*-tests were used to analyze all data in tumor bearing mice (i.e., Exp. 2 and 3), with the exception of Fig. 5A (Mixed-effects analysis). A two-way ANOVA was used to analyze all data in non-tumor bearing mice (i.e., Exp. 1). Post-hoc analysis was performed using Fisher's LSD tests. For all mean comparisons, $p < 0.05$ was considered statistically significant. All statistical analyses were performed using GraphPad Prism 9.5 software.

Ethics statement

All experiments were approved and conducted in accordance with guidelines set by the West Virginia University Institutional Animal Care and Use Committee Protocol #1801012111.

Data availability

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

CRediT authorship contribution statement

William H. Walker II: Writing - review & editing, Writing - original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Jennifer A. Liu:** Writing - review & editing, Methodology, Investigation. **O. Hecmarie Meléndez-Fernández:** Writing - review & editing, Methodology, Investigation. **Laura E. May:** Writing - review & editing, Methodology, Investigation. **Claire O. Kisamore:** Writing - original draft, Methodology, Investigation. **Kathleen M. Brundage:** Writing - review & editing, Methodology, Investigation, Conceptualization. **Randy J. Nelson:** Writing - review & editing, Writing - original draft, Supervision, Funding acquisition, Conceptualization. **A. Courtney DeVries:** Writing - review & editing, Writing - original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23366.

References

[1] M. Ono, J.M. Ogilvie, J. Wilson, H. Green, S. Chambers, T. Ownsworth, D.H.K. Shum, A meta-analysis of cognitive impairment and decline associated with adjuvant chemotherapy in women with breast cancer, Front. Oncol. 5 (2015), https://doi.org/10.3389/FONC.2015.00059/TEXT.

[2] K. Tsaras, I.V. Papathanasiou, D. Mitsi, A. Veneti, M. Kelesi, S. Zyga, E.C. Fradelos, Assessment of depression and anxiety in breast cancer patients: prevalence and associated factors, Asian Pac. J. Cancer Prev. APJCP 19 (2018) 1661, https://doi.org/10.22034/APJCP.2018.19.6.1661.

[3] W.H. Walker, J.C. Borniger, Molecular mechanisms of cancer-induced sleep disruption, 2019, Int. J. Mol. Sci. 20 (2019), https://doi.org/10.3390/IJMS20112780, 2780 20, 2780.

[4] X. Wang, N. Wang, L. Zhong, S. Wang, Y. Zheng, B. Yang, J. Zhang, Y. Lin, Z. Wang, Prognostic value of depression and anxiety on breast cancer recurrence and mortality: a systematic review and meta-analysis of 282,203 patients, Mol. Psychiatr. 2512 (25) (2020) 3186–3197, https://doi.org/10.1038/s41380-020-00865-6, 2020.

[5] J.C. Borniger, W.H. Walker Ii, K.M. Surbhi Emmer, N. Zhang, A.A. Zalenski, S.L. Muscarella, J.A. Fitzgerald, A.N. Smith, C.J. Braam, T. TinKai, U.J. Magalang, M.B. Lustberg, R.J. Nelson, A.C. DeVries, A role for hypocretin/orexin in metabolic and sleep abnormalities in a mouse model of non-metastatic breast cancer, Cell Metabol. 0 (2018), https://doi.org/10.1016/j.cmet.2018.04.021.

[6] L.M. Pyter, S.F. Cochrane, R.L. Ouwenga, P.N. Patel, V. Pineros, B.J. Prendergast, Mammary tumors induce select cognitive impairments, Brain Behav. Immun. 24 (2010) 903–907, https://doi.org/10.1016/j.bbi.2010.02.004.

[7] L.M. Pyter, V. Pineros, J. a Galang, M.K. McClintock, B.J. Prendergast, Peripheral tumors induce depressive-like behaviors and cytokine production and alter hypothalamic-pituitary-adrenal axis regulation, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 9069–9074, https://doi.org/10.1073/pnas.0811949106.

[8] G. Winocur, H. Berman, M. Nguyen, M.A. Binns, M. Henkelman, M. van Eede, M. Piquette-Miller, M.J. Sekeres, J.M. Wojtowicz, J. Yu, H. Zhang, I.F. Tannock, Neurobiological mechanisms of chemotherapy-induced cognitive impairment in a transgenic model of breast cancer, Neuroscience 369 (2018) 51–65, https://doi.org/10.1016/J.NEUROSCIENCE.2017.10.048.

[9] Y. Alatawi, R.A. Hansen, C. Chou, J. Qian, V. Suppiramaniam, G. Cao, The impact of cognitive impairment on survival and medication adherence among older women with breast cancer, Breast Cancer 28 (2021) 277–288, https://doi.org/10.1007/S12282-020-01155-3/TABLES/5.

[10] O. Palesh, A. Aldridge-Gerry, J.M. Zeitzer, C. Koopman, E. Neri, J. Giese-Davis, B. Jo, H. Kraemer, B. Nouriani, D. Spiegel, Actigraphy-measured sleep disruption as a predictor of survival among women with advanced breast cancer, Sleep 37 (2014) 837–842, https://doi.org/10.5665/sleep.3642.

[11] A. Soucise, C. Vaughn, C.L. Thompson, A.E. Millen, J.L. Freudenheim, J. Wactawski-Wende, A.I. Phipps, L. Hale, L. Qi, H.M. Ochs-Balcom, Sleep quality, duration, and breast cancer aggressiveness, Breast Cancer Res. Treat. 164 (2017) 169–178, https://doi.org/10.1007/S10549-017-4245-1/TABLES/4.

[12] S. Ancoli-Israel, L. Liu, M. Rissling, L. Natarajan, A.B. Neikrug, B.W. Palmer, P.J. Mills, B.A. Parker, G.R. Sadler, J. Maglione, Sleep, fatigue, depression, and circadian activity rhythms in women with breast cancer before and after treatment: a 1-year longitudinal study, Support. Care Cancer 22 (2014) 2535–2545, https://doi.org/10.1007/S00520-014-2204-5/FIGURES/4.

[13] A.R. Costa, F. Fontes, S. Pereira, M. Gonçalves, A. Azevedo, N. Lunet, Impact of breast cancer treatments on sleep disturbances – a systematic review, Breast 23 (2014) 697–709, https://doi.org/10.1016/J.BREAST.2014.09.003.

[14] C. Farrell, S.G. Brearley, M. Pilling, A. Molassiotis, The impact of chemotherapy-related nausea on patients' nutritional status, psychological distress and quality of life. Support, Care Cancer 21 (2013) 59–66, https://doi.org/10.1007/S00520-012-1493-9/TABLES/2.

[15] E.Y. Ibrahim, I. Domenicano, K. Nyhan, M. Elfil, S.S. Mougalian, B. Cartmel, B.E. Ehrlich, Cognitive effects and depression associated with taxane-based chemotherapy in breast cancer survivors: a meta-analysis, Front. Oncol. 11 (2021) 741, https://doi.org/10.3389/FONC.2021.642382/BIBTEX.

[16] K.D. Hodgson, A.D. Hutchinson, C.J. Wilson, T. Nettelbeck, A meta-analysis of the effects of chemotherapy on cognition in patients with cancer, Cancer Treat Rev. 39 (2013) 297–304, https://doi.org/10.1016/J.CTRV.2012.11.001.

[17] J.C. Borniger, M.M. Gaudier-Diaz, N. Zhang, R.J. Nelson, A.C. DeVries, Cytotoxic chemotherapy increases sleep and sleep fragmentation in non-tumor-bearing mice, Brain Behav. Immun., Sleep, Brain, Behavior, and Immunity 47 (2015) 218–227, https://doi.org/10.1016/j.bbi.2014.11.001.

[18] A. Chang, N.C. Chung, A.J. Lawther, A.I. Ziegler, D.M. Shackleford, E.K. Sloan, A.K. Walker, The anti-inflammatory drug aspirin does not protect against chemotherapy-induced memory impairment by paclitaxel in mice, Front. Oncol. 10 (2020) 2810, https://doi.org/10.3389/FONC.2020.564965/BIBTEX.

[19] A.C.A. Chiang, X. Huo, A. Kavelaars, C.J. Heijnen, Chemotherapy accelerates age-related development of tauopathy and results in loss of synaptic integrity and cognitive impairment, Brain Behav. Immun. 79 (2019) 319–325, https://doi.org/10.1016/J.BBI.2019.04.005.

[20] D.D. Shi, Y.H. Huang, C.S.W. Lai, C.M. Dong, L.C. Ho, E.X. Wu, Q. Li, X.M. Wang, S.K. Chung, P.C. Sham, Z.J. Zhang, Chemotherapy-induced cognitive impairment is associated with cytokine dysregulation and disruptions in neuroplasticity, Mol. Neurobiol. 56 (2019) 2234–2243, https://doi.org/10.1007/S12035-018-1224-4/TABLES/2.

[21] W. Toma, S.L. Kyte, D. Bagdas, Y. Alkhlaif, S.D. Alsharari, A.H. Lichtman, Z.J. Chen, E. Del Fabbro, J.W. Bigbee, D.A. Gewirtz, M.I. Damaj, Effects of paclitaxel on the development of neuropathy and affective behaviors in the mouse, Neuropharmacology 117 (2017) 305–315, https://doi.org/10.1016/J.NEUROPHARM.2017.02.020.

[22] M. Egeland, C. Guinaudie, A. Du Preez, K. Musaelyan, P.A. Zunszain, C. Fernandes, C.M. Pariante, S. Thuret, Depletion of adult neurogenesis using the chemotherapy drug temozolomide in mice induces behavioural and biological changes relevant to depression, Transl. Psychiatry 7 (2017), https://doi.org/10.1038/tp.2017.68.

[23] O. Palesh, Peppone Innominato Janelsins, M. Jeong, L. Sprod, R.M. Savard Rotatori, Telli Mustian Kesler, Prevalence, putative mechanisms, and current management of sleep problems during chemotherapy for cancer, Nat. Sci. Sleep 151 (2012), https://doi.org/10.2147/nss.s18895.

[24] R. Seigers, J.E. Fardell, Neurobiological basis of chemotherapy-induced cognitive impairment: a review of rodent research, Neurosci. Biobehav. Rev. (2011), https://doi.org/10.1016/j.neubiorev.2010.09.006.

[25] H.R. Smith, Depression in cancer patients: Pathogenesis, implications and treatment, Oncol. Lett. 9 (2015) 1509–1514, https://doi.org/10.3892/OL.2015.2944 (review).

[26] E.G. Vichaya, G.S. Chiu, K. Krukowski, T.E. Lacourt, A. Kavelaars, R. Dantzer, C.J. Heijnen, A.K. Walker, Mechanisms of chemotherapy-induced behavioral toxicities, Front. Neurosci. 9 (2015) 131, https://doi.org/10.3389/FNINS.2015.00131/BIBTEX.

[27] J.C. Santos, L.M. Pyter, Neuroimmunology of behavioral comorbidities associated with cancer and cancer treatments, Front. Immunol. 9 (2018) 1195, https://doi.org/10.3389/fimmu.2018.01195.

[28] S. Hughes, L.M. Jaremka, C.M. Alfano, R. Glaser, S.P. Povoski, A.M. Lipari, D.M. Agnese, W.B. Farrar, L.D. Yee, W.E. Carson, W.B. Malarkey, J.K. Kiecolt-Glaser, Social support predicts inflammation, pain, and depressive symptoms: longitudinal relationships among breast cancer survivors, Psychoneuroendocrinology 42 (2014) 38–44, https://doi.org/10.1016/j.psyneuen.2013.12.016.

[29] E. Maunsell, J. Brisson, L. Deschênes, Social support and survival among women with breast cancer, Cancer 76 (1995) 631–637, https://doi.org/10.1002/1097-0142(19950815)76:4<631::AID-CNCR2820760414>3.0.CO, 2-9.

[30] B. Nausheen, Y. Gidron, R. Peveler, R. Moss-Morris, Social support and cancer progression: a systematic review, J. Psychosom. Res. 67 (2009) 403–415, https://doi.org/10.1016/J.JPSYCHORES.2008.12.012.

[31] A. Wondimagegnehu, W. Abebe, A. Abraha, S. Teferra, Depression and social support among breast cancer patients in Addis Ababa, Ethiopia, BMC Cancer 19 (2019) 1–8, https://doi.org/10.1186/S12885-019-6007-4/TABLES/2.

[32] H. Zamanian, M. Amini-Tehrani, Z. Jalali, M. Daryaafzoon, S. Ala, S. Tabrizian, S. Foroozanfar, Perceived social support, coping strategies, anxiety and depression among women with breast cancer: evaluation of a mediation model, Eur. J. Oncol. Nurs. 50 (2021), 101892, https://doi.org/10.1016/J.EJON.2020.101892.

[33] C.H. Kroenke, L.D. Kubzansky, E.S. Schernhammer, M.D. Holmes, I. Kawachi, Social networks, social support, and survival after breast cancer diagnosis, J. Clin. Oncol. 24 (2006) 1105–1111, https://doi.org/10.1200/JCO.2005.04.2846.

[34] A. Montazeri, S. Jarvandi, S. Haghighat, M. Vahdani, A. Sajadian, M. Ebrahimi, M. Haji-Mahmoodi, Anxiety and depression in breast cancer patients before and after participation in a cancer support group, Patient Educ. Couns. 45 (2001) 195–198, https://doi.org/10.1016/S0738-3991(01)00121-5.

[35] A.F. Chou, S.L. Stewart, R.C. Wild, J.R. Bloom, Social support and survival in young women with breast carcinoma, Psycho Oncol. 21 (2012) 125–133, https://doi.org/10.1002/PON.1863.

[36] W.H. Walker II, O.H. Meléndez-Fernández, J.L. Pascoe, N. Zhang, A.C. Devries, Social enrichment attenuates chemotherapy induced pro-inflammatory cytokine production and affective behavior via oxytocin signaling, Brain Behav. Immun. 89 (2020) 451–464, https://doi.org/10.1016/j.bbi.2020.07.032.

[37] R.M. Ritzel, S.J. Doran, E.P. Glaser, V.E. Meadows, A.I. Faden, B.A. Stoica, D.J. Loane, Old age increases microglial senescence, exacerbates secondary neuroinflammation, and worsens neurological outcomes after acute traumatic brain injury in mice, Neurobiol. Aging 77 (2019) 194–206, https://doi.org/10.1016/J.NEUROBIOLAGING.2019.02.010.

[38] A. Berry, B. Collacchi, S. Capoccia, M.T. D'Urso, S. Cecchetti, C. Raggi, P. Sestili, E. Aricò, G. Pontecorvi, R. Puglisi, E. Ortona, F. Cirulli, Chronic isolation stress affects central neuroendocrine signaling leading to a metabolically active microenvironment in a mouse model of breast cancer, Front. Behav. Neurosci. 15 (2021) 141, https://doi.org/10.3389/FNBEH.2021.660738/BIBTEX.

[39] E.M. Gibson, S. Nagaraja, A. Ocampo, L.T. Tam, L.S. Wood, P.N. Pallegar, J.J. Greene, A.C. Geraghty, A.K. Goldstein, L. Ni, P.J. Woo, B.A. Barres, S. Liddelow, H. Vogel, M. Monje, Methotrexate chemotherapy induces persistent tri-glial dysregulation that underlies chemotherapy-related cognitive impairment, Cell 176 (2019) 43–55.e13, https://doi.org/10.1016/J.CELL.2018.10.049.
[40] R. Seigers, J. Timmermans, H.J. van der Horn, E.F.J. de Vries, R.A. Dierckx, L. Visser, S.B. Schagen, F.S.A.M. van Dam, J.M. Koolhaas, B. Buwalda, Methotrexate reduces hippocampal blood vessel density and activates microglia in rats but does not elevate central cytokine release, Behav. Brain Res. 207 (2010) 265–272, https://doi.org/10.1016/j.bbr.2009.10.009.
[41] C.V. Grant, K.A. Sullivan, K.M. Wentworth, L.D. Otto, L.D. Strehle, J.J. Otero, L.M. Pyter, Microglia are implicated in the development of paclitaxel chemotherapy-associated cognitive impairment in female mice, Brain Behav. Immun. 108 (2023) 221–232, https://doi.org/10.1016/J.BBI.2022.12.004.
[42] A. Ożańska, D. Szymczak, J. Rybka, Pattern of human monocyte subpopulations in health and disease, Scand. J. Immunol. 92 (2020), e12883, https://doi.org/10.1111/SJI.12883.
[43] S. Gordon, P.R. Taylor, Monocyte and macrophage heterogeneity, Nat. Rev. Immunol. 512 (5) (2005) 953–964, https://doi.org/10.1038/nri1733, 2005.
[44] E. Brandt, G. Tane Woerly, A. Younes, S. Ben Loiseau, M. Capron, IL-4 production by human polymorphonuclear neutrophils, J. Leukoc. Biol. 68 (2000) 125–130, https://doi.org/10.1189/JLB.68.1.125.
[45] L. Liu, P.J. Mills, M. Rissling, L. Fiorentino, L. Natarajan, J.E. Dimsdale, G.R. Sadler, B.A. Parker, S. Ancoli-Israel, Fatigue and sleep quality are associated with changes in inflammatory markers in breast cancer patients undergoing chemotherapy, Brain Behav. Immun. (2012), https://doi.org/10.1016/j.bbi.2012.02.001.
[46] R.T. Penson, K. Kronish, Z. Duan, A.J. Feller, P. Stark, S.E. Cook, L.R. Duska, A.F. Fuller, A.K. Goodman, N. Nikrui, K.M. Macneill, U.A. Matulonis, F.I. Preffer, M. V. Seiden, Cytokines IL-1β, IL-2, IL-6, IL-8, MCP-1, GM-CSF and TNFα in patients with epithelial ovarian cancer and their relationship to treatment with paclitaxel, Int. J. Gynecol. Cancer 10 (2000) 33–41, https://doi.org/10.1046/j.1525-1438.2000.00003.x.
[47] L. Pusztai, T.R. Mendoza, J.M. Reuben, M.M. Martinez, J.S. Willey, J. Lara, A. Syed, H.A. Fritsche, E. Bruera, D. Booser, V. Valero, B. Arun, N. Ibrahim, E. Rivera, M. Royce, C.S. Cleeland, G.N. Hortobagyi, Changes in plasma levels of inflammatory cytokines in response to paclitaxel chemotherapy, Cytokine 25 (2004) 94–102, https://doi.org/10.1016/j.cyto.2003.10.004.
[48] S. Kesler, M. Janelsins, D. Koovakkattu, O. Palesh, K. Mustian, G. Morrow, F.S. Dhabhar, Reduced hippocampal volume and verbal memory performance associated with interleukin-6 and tumor necrosis factor-alpha levels in chemotherapy-treated breast cancer survivors, Brain Behav. Immun. 30 (2013), https://doi.org/10.1016/j.bbi.2012.05.017.
[49] S.K. Lutgendorf, A.Z. Weinrib, F. Penedo, D. Russell, K. DeGeest, E.S. Costanzo, P.J. Henderson, S.E. Sephton, N. Rohleder, J.A. Lucci, S. Cole, A.K. Sood, D. M. Lubaroff, Interleukin-6, cortisol, and depressive symptoms in ovarian cancer patients, J. Clin. Oncol. 26 (2008) 4820–4827, https://doi.org/10.1200/JCO.2007.14.1978.
[50] R.A. Budiu, A.M. Vlad, L. Nazario, C. Bathula, K.L. Cooper, J. Edmed, P.H. Thaker, J. Urban, P. Kalinski, A.V. Lee, E.L. Elishaev, T.P. Conrads, M.S. Flint, Restraint and social isolation stressors differentially regulate adaptive immunity and tumor angiogenesis in a breast cancer mouse model, Cancer Clin. Oncol. 6 (2017) 12, https://doi.org/10.5539/CCO.V6N1P12.
[51] G.L. Hermes, B. Delgado, M. Tretiakova, S.A. Cavigelli, T. Krausz, S.D. Conzen, M.K. McClintock, Social isolation dysregulates endocrine and behavioral stress while increasing malignant burden of spontaneous mammary tumors, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 22393–22398, https://doi.org/10.1073/PNAS.0910753106/SUPPL_FILE/0910753106SI.PDF.
[52] J. Faraji, M. Karimi, N. Soltanpour, A. Moharrerie, Z. Rouhzadeh, H. Lotfi, S.A. Hosseini, S.Y. Jafari, S. Roudaki, R. Moeeini, G.A.S. Metz, Oxytocin-mediated social enrichment promotes longer telomeres and novelty seeking, Elife 7 (2018), https://doi.org/10.7554/ELIFE.40262.
[53] H. Liu, C.W. Gruber, P.F. Alewood, A. Möller, M. Muttenthaler, The oxytocin receptor signalling system and breast cancer: a critical review, Oncogene 3937 (39) (2020) 5917–5932, https://doi.org/10.1038/s41388-020-01415-8, 2020.
[54] C. Sue Carter, W.M. Kenkel, E.L. Maclean, S.R. Wilson, A.M. Perkeybile, J.R. Yee, C.F. Ferris, H.P. Nazarloo, S.W. Porges, J.M. Davis, J.J. Connelly, M. A. Kingsbury, Is oxytocin "nature's medicine", Pharmacol. Rev. 72 (2020) 829–861, https://doi.org/10.1124/PR.120.019398.
[55] E. Díaz-del Cerro, N. Ceprián, J. Félix, M. De la Fuente, A short social interaction between adult and old mice improves the homeostatic systems and increases healthy longevity, Exp. Gerontol. 158 (2022), 111653, https://doi.org/10.1016/J.EXGER.2021.111653.
[56] D. Meirow, J. Levron, T. Eldar-Geva, I. Hardan, E. Fridman, Y. Zalel, E. Schiff, J. Dor, Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy [5], N. Engl. J. Med. (2005), https://doi.org/10.1056/NEJMc055237.
[57] D. Meirow, G. Assad, J. Dor, J. Rabinovici, The GnRh antagonist cetrorelix reduces cyclophosphamide-induced ovarian follicular destruction in mice, Hum. Reprod. 19 (2004) 1294–1299, https://doi.org/10.1093/humrep/deh257.
[58] S. Reagan-Shaw, M. Nihal, N. Ahmad, Dose translation from animal to human studies revisited, FASEB J. 22 (3) (2008) 659–666, https://doi.org/10.1096/fj.07-9574LSF.
[59] M.M. Tomayko, C.P. Reynolds, Determination of subcutaneous tumor size in athymic (nude) mice, Cancer Chemother. Pharmacol. 24 (1989) 148–154, https://doi.org/10.1007/BF00300234.
[60] G.H. Heppner, F.R. Miller, P.M. Shekhar, Nontransgenic models of breast cancer, Breast Cancer Res. 2 (2000) 331–334, https://doi.org/10.1186/bcr77.
[61] B. Korin, T. Dubovik, A. Rolls, Mass cytometry analysis of immune cells in the brain, Nat. Protoc. 132 13 (2018) 377–391, https://doi.org/10.1038/nprot.2017.155, 2018.