Published in final edited form as:

Behav Brain Res. 2007 July 19; 181(1): 168–172. doi:10.1016/j.bbr.2007.04.003.

Cancer chemotherapy impairs contextual but not cue-specific fear memory

Jill E. MacLeod^a, Joyce A. DeLeo^b, William F. Hickey^c, Tim A. Ahles^{d,1}, Andrew J. Saykin^{d,2}, and David J. Bucci^{a,*}

^aDepartment of Psychological and Brain Sciences, Dartmouth College, Hanover, NH 03755, United States

^bDepartment of Anesthesiology, Dartmouth Medical School, Lebanon, NH 03756, United States

^cDepartment of Pathology, Dartmouth Medical School, Lebanon, NH 03756, United States

^dDepartment of Psychiatry, Dartmouth Medical School, Lebanon, NH 03756, United States

Abstract

This study examined the effects of a standard breast cancer chemotherapeutic protocol on learning and memory in rats. Ovariectomized rats were treated once a week for 3 weeks with a combination of cyclophosphamide and doxorubicin prior to training in a classical fear conditioning task.

Training took place 1 week after the final treatment. During the training session, an auditory stimulus (a tone) was paired with a mild foot-shock. The resulting conditioned fear to the tone (cue-specific fear) and to the training environment (contextual fear) was measured in subsequent test sessions. Chemotherapy did not affect the acquisition of the conditioned response (freezing) during the training session or the expression of fear during the tone test session. In contrast, rats treated with cyclophosphamide and doxorubicin exhibited decreased freezing during the context test session, suggestive of a specific deficit in hippocampal-related learning and memory.

Together, these data indicate that administration of cyclophosphamide and doxorubicin may have toxic effects on the hippocampus and results in specific learning deficits shortly after treatment has ended.

Keywords

Cyclophosphamide; Doxorubicin; Chemotherapy; Learning; Rat

A growing body of evidence indicates that cancer chemotherapy results in cognitive changes during treatment, immediately post-treatment, and up to 10 years following therapy [2,4,15,21,25,32]. The cognitive deficits that are experienced are diverse and vary in severity; however, problems with memory function and executive processes are the most

© 2007 Elsevier B.V. All rights reserved.

*Corresponding author at: Department of Psychological and Brain Sciences, Dartmouth College, 6207 Moore Hall, Hanover, NH 03755, United States. Tel.: +1 603 646 3439; fax: +1 603 646 1419. david.j.bucci@dartmouth.edu (D.J. Bucci).

¹Current address: Neurocognitive Research Laboratory, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, United States.

²Current address: Department of Radiology, Indiana University School of Medicine, Indianapolis, IN 46202.

common. These impairments can have a negative impact on patients' quality of life and pose a significant challenge for people with cancer [10]. Nevertheless, there is surprisingly little known about which cytotoxic agents or combinations of agents produce cognitive impairment. Moreover, the neural mechanism(s) by which chemotherapy produces cognitive decline is unclear, as are the specific cognitive domains that are affected.

Studying the effects of chemotherapy on cognitive function in laboratory animals may be particularly useful in addressing these issues. Animal models provide significant control over the subjects' health history, thereby reducing the potential confound of co-occurring disease and/or co-existing psychological factors. In addition, a great deal is already known about the neural systems that underlie learning and memory, particularly in rats, allowing one to make valuable predictions about the potential locus of action of pharmacological agents. Likewise, examining the effects of chemotherapy on cognition in rats can be combined with various histological methods to identify the neurobiological effects of different agents. Despite these advantages very few studies have made use of animal models to investigate the effects of chemotherapy on cognitive function [18,24,35].

The present study thus examined the effects of a standard breast cancer chemotherapeutic protocol on the ability of female rats to learn and remember new information soon after a treatment regimen (i.e., anterograde memory). Ovariectomized rats were used instead of intact females because chemotherapy has been shown to reduce estrogen levels [14]. This would have resulted in a potential confound since estrogen affects cognitive function [11]. In addition, chemotherapy also induces menopause in women, and may thus also alter the estrous cycle in rats.

Thirty rats were obtained at 8 weeks of age from Harlan Sprague–Dawley (Indianapolis, IN) and housed in pairs upon arrival. Rats were maintained on a 12 h light/dark cycle with free access to food and water in accordance with IACUC-approved protocols and AAALAC guidelines. Each rat in the chemotherapy group ($n = 15$) was treated once a week for 3 weeks with a combination of cyclophosphamide (40 mg/kg) and doxorubicin (4 mg/kg; Sigma–Aldrich, Co.). Prior to each tail vein injection, rats were anesthetized with isoflurane (2–3% in oxygen) and warm saline (1 ml) was flushed through polypropylene tubing connected to a 25 gauge, 3/4-in. butterfly needle. A syringe containing the appropriate doxorubicin dose was then attached to the tubing and slowly administered, followed by 1 ml saline. Cyclophosphamide was then administered in the same manner; vehicle-treated rats ($n = 15$) received 3.5 ml of warm saline. Rats were allowed 1 week to recover prior to the start of the behavioral procedures. This treatment schedule and dosing regimen was based on preliminary studies to develop a chemotherapy-induced cognitive deficit model in rats using human drug dosing concentrations; similar procedures have also been reported in a recent study [35].

All behavioral procedures took place in standard rat operant conditioning chambers (Med Associates, St. Albans, VT). Scrambled alternating current was delivered through the grid floor by a constant current shock source. A speaker connected to a programmable audio input generator was located at the top right corner of the front panel of the chamber and was used to deliver the 1500 Hz, 86 dB auditory stimulus. A partially shaded houselight (28

V, 100 mA) mounted centrally at the top of the front wall illuminated the chamber during training and testing. Each chamber was located in a sound-attenuating cubicle and a video camera mounted on the outside of the back wall provided full view of the rat in the entire chamber.

Rats were trained in a signaled fear conditioning paradigm used previously [3,20]. During the training session, rats received three trials each consisting of a 10-s presentation of the tone followed immediately by footshock (1 mA, 1 s). The trials began 2 min after the rats were placed in the chambers and were separated by 64 s. Rats were returned to their home cages 64 s after the last trial. Contextual fear memory was tested 24 h later by placing the rats back in the conditioning chamber for a 10-min test session during which no tones or shocks were presented. Cued fear memory was tested 24 h after the context test by re-exposing the rats to the tone (twenty 10-s trials) in a new context (no shock was delivered). This paradigm is particularly useful since the neural circuitry underlying cue-specific and contextual conditioned fear is already well established [20]. In addition, since training takes place in a single session and is followed by distinct test sessions, it is possible to gain insight into the specific phases and nature of mnemonic function that may be affected.

Freezing behavior served as the index of conditioned fear and was defined as total motor immobility except for breathing. During the training session, behavior was recorded every 8 s during the 64-s period after each training trial (i.e., post-shock freezing). The context test session was divided into 64-s epochs observation periods and freezing was scored every 8 s. For the cue test session, freezing was recorded every 2 s during each 10-s presentation of the tone. The frequency of freezing behavior was converted to a percentage of total observations. For the training session, the amount of freezing observed in the vehicle and drug groups after the final training trial was analyzed using an independent measures *t*-test. Data from the context and tone test sessions were analyzed using repeated measures analysis of variance (ANOVA) with group as the between-subjects factor and 64-s epoch or trial as the within-subjects factor, respectively. An alpha level of 0.05 was used for all analyses.

Prior to the start of treatment, both sets of rats were healthy and had similar mean body weights (237 ± 3 and 231 ± 2 g for the rats that would receive saline or drug, respectively). By the end of the third treatment, all rats receiving cyclophosphamide and doxorubicin exhibited alopecia and had gained less weight over the 3-week treatment period compared to controls (mean body weights were 245 ± 5 and 285 ± 4 g, respectively), suggesting that chemotherapy did have the expected toxic effect. By the start of training, all but three rats in the chemotherapy group had returned to their pretraining body weights or more. There were no behavioral difference noted between these three rats and the other rats in the drug-treatment group ($p > 0.3$).

The amount of post-shock freezing observed during the training session was comparable between drug-treated and vehicle-treated rats [$t(28) = -0.9, p > 0.4$] indicating that cyclophosphamide and doxorubicin did not significantly affect acquisition of the conditioned freezing response. The mean percent freezing behavior observed in the vehicle-treated and drug-treated groups was $76 \pm 4\%$ and $71 \pm 5\%$, respectively. During the context test session, drug-treated rats exhibited less conditioned freezing than vehicle-treated rats, as

shown in Fig. 1. This was confirmed by a repeated measures ANOVA that revealed a significant main effect of group [$F(1, 28) = 4.2, p < 0.05$]. There was no significant interaction between group and 64-s epoch ($p > 0.9$). There was a main effect of epoch, however, confirming that rats in both groups exhibited less freezing as the context test session continued [$F(9, 252) = 6.8, p < 0.001$]. In contrast to the effects observed on contextual fear memory, drug-treatment did not affect conditioned freezing during the tone test session, as illustrated in Fig. 2. Indeed, there was no main effect of group ($p > 0.9$) and no significant group $\times$ trial interaction ($p > 0.4$). Freezing behavior did decrease across trials [main effect of trial, $F(19, 532) = 9.2, p < 0.001$] as is typically observed when the tone is no longer paired with shock (i.e., extinction).

These data indicate that treatment with cyclophosphamide and doxorubicin impairs memory for specific types of information learned shortly after treatment. The specific deficits in contextual fear memory observed in this study suggest that chemotherapy may have detrimental effects on the hippocampus. Indeed, previous studies provide substantial evidence that the hippocampus plays a critical role in contextual fear conditioning [1,7,13,19,20]. In a signaled shock preparation such as the one used here, in which a discrete stimulus precedes the occurrence of footshock, contextual conditioning has been shown to be particularly sensitive to hippocampal damage [23]. An effect on hippocampal-related learning and memory is consistent with the recent observation that chemotherapeutic agents can increase cell death and decrease neurogenesis particularly in the dentate gyrus of the hippocampus [9].

That the hippocampus may be especially sensitive to the effects of chemotherapy is not surprising. It is well established that the hippocampus is preferentially susceptible to the effects of environmental toxins [29], ischemia [27], and radiation [5]. In addition to causing cell death in the hippocampus, a variety of insults also result in extensive remodeling of hippocampal synapses [8]. In the case of chemotherapy, many investigators initially thought that antineoplastic agents had little ability to penetrate the blood brain barrier, however, more recent studies have indicated higher than expected concentrations in CSF and brain tissue [30,31]. Although the specific pathophysiological mechanisms leading to cognitive deficits are not well understood, these agents can produce a variety of neurotoxic effects [16,31,33,34]. In addition, our group has completed a pilot investigation using structural and functional MRI to study long-term survivors of breast cancer who had received systemic chemotherapy [25]. The results suggest that chemotherapy may be associated with structural and functional changes, in agreement with other studies reporting changes in white matter following chemotherapy treatment [28].

The decrease in expression of contextual fear in the group receiving chemotherapy likely reflects a deficit in retaining contextual information. This could be the result of impaired memory consolidation that normally occurs after the training experience and depends on the hippocampus [17,20] or poor acquisition of contextual information during the training phase. Another interpretation is that the decrease in freezing reflects enhanced extinction of contextual fear. However, this is unlikely since the level of freezing is lower in drug-treated rats compared to controls from the outset of the context test session and remains lower throughout the entire test period. In addition, the extinction curves are similar in drug-treated

and control rats. The reduction in freezing is also not likely due to a simple performance deficit since rats treated with cyclophosphamide and doxorubicin exhibited normal levels of freezing during training and during the tone test session. Group differences in contextual freezing also cannot be attributed to a difference in levels of circulating sex hormones between chemotherapy-treated and vehicle-treated rats since all rats were ovariectomized prior to the start of the experiment.

To our knowledge, only a few published studies have examined the effects of chemotherapy on cognition in rats. In a recent study [35], it was reported that treatment with methotrexate and 5-fluorouracil, two other commonly used cancer chemotherapy agents, produced deficits in spatial as well as non-spatial memory. These results are consistent with the present findings, and interestingly, the study employed the same drug regimen (i.e., once per week for 3 weeks) and recovery period as our study. In a brief letter to the editor published by Reiriz et al. [24], the authors reported that cyclophosphamide administration impaired memory in an inhibitory avoidance task in mice. This is a hippocampal-dependent task, and like the present study, involves aversive conditioning. Interestingly, memory was impaired when a single dose of cyclophosphamide was administered 1 day before training, but not 1 week before training. Although the lack of effect with the latter treatment time differs from the present findings, it is possible that similar results would have been obtained had Reiriz et al. used multiple injections.

In a third study, cyclophosphamide or 5-fluorouracil was administered to female rats, which were subsequently tested for spatial learning ability in a Morris water maze task and a T-maze active avoidance task [18]. In contrast to the present findings, rats treated with either drug exhibited enhanced performance in these tasks. However, there are several important differences between our study and that of Lee et al. that might account for the disparate results. First, in the study by Lee et al. rats were trained 7–9 weeks or 29–42 weeks after chemotherapy, which is much later than the 1 week recovery time used in the present study. Thus the two studies were focusing on cognitive effects at distinctly different times after treatment. It is also important to note, as Lee et al. suggest, that different results might be expected when multiple drugs are administered simultaneously as opposed to individually. In our study it was important to administer cyclophosphamide and doxorubicin together to most accurately model treatment regimens in women with breast cancer. Other differences between the two studies that could likely lead to differing results include the age of the subjects as well as the fact that the female rats in Lee et al.'s study were not ovariectomized, leaving open the possible influence and interaction of cycling hormones with chemotherapy. Indeed, the same chemotherapeutic agents used in these studies have been shown to reduce estrogen levels [14], and lower estrogen has been associated with improved performance in spatial tasks [11]. Lastly, it is important to note different components of the hippocampal system appear to be involved in the spatial tasks used by Lee et al. and the contextual conditioning procedures used in our study [6,12].

The observation of impaired memory in the present study and the ones by Winocur et al. [35] and Reiriz et al. [24] is consistent with a growing number of clinical reports of impaired learning and memory after chemotherapy [2,22,26]. However, it is clear that additional studies are needed to fully characterize the effects of chemotherapeutic agents on learning

and memory in rat models. Animal studies will be particularly useful in establishing the time course of cognitive decline following treatment, as well as the mechanisms by which chemotherapy produces deficits. Complementary studies in rats with experimental tumors will also need to be carried out to examine the influence of a tumor state on the specific effect of chemotherapy on cognition.

Acknowledgments

Research supported by a grant from the Neuroscience Center at Dartmouth.

References

1. Anagnostaras SG, Gale GD, Fanselow MS. Hippocampus and contextual fear conditioning: recent controversies and advances. *Hippocampus*. 2001; 11:8–17. [PubMed: 11261775]
2. Ahles TA, Saykin AJ, Furstenberg CT, Cole B, Mott LA, Skalla K, et al. Neuropsychological impact of standard-dose chemotherapy in long-term survivors of breast cancer and lymphoma. *J Clin Oncol*. 2002; 20:485–93. [PubMed: 11786578]
3. Arenos JD, Musty RE, Bucci DJ. Blockade of cannabinoid CB1 receptors alters contextual learning and memory. *Eur J Pharmacol*. 2006; 539:177–83. [PubMed: 16716290]
4. Brezden C, Philips K, Abdoell M. Cognitive function in breast cancer patients receiving adjuvant chemotherapy. *J Clin Oncol*. 2000; 18:2695–701. [PubMed: 10894868]
5. Brizzee KR, Ordly JM, Kaack MB, Beavers T. Effect of prenatal ionizing radiation on the visual cortex and hippocampus of newborn squirrel monkeys. *J Neuropathol Exp Neurol*. 1980; 39:523–40. [PubMed: 7217998]
6. Burwell RD, Saddoris MP, Bucci DJ, Wiig KA. Corticohippocampal contributions to spatial and contextual learning. *J Neurosci*. 2004; 24:3826–36. [PubMed: 15084664]
7. Corcoran KA, Maren S. Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. *J Neurosci*. 2001; 21:1720–6. [PubMed: 11222661]
8. Crepel V, Epsztein J, Ben-Ari Y. Ischemia induces short- and long-term remodeling of synaptic activity in the hippocampus. *J Cell Mol Med*. 2003; 7:401–7. [PubMed: 14754508]
9. Dietrich J, Han R, Yang Y, Mayer-Proschel M, Noble M. CNS progenitor cells and oligodendrocytes are targets of chemotherapeutic agents in vitro and in vivo. *J Biol*. 2006; 5(22):1–23. [PubMed: 16515716]
10. Ferrell B, Hassey–Dow K. Quality of life among long-term cancer survivors. *Oncology*. 1997; 11:565–76. [PubMed: 9130276]
11. Galea LA, Kavaliers M, Ossenkopp KP, Hampson E. Gonadal hormone levels and spatial learning performance in the Morris water maze in male and female meadow voles, *Microtus pennsylvanicus*. *Horm Behav*. 1995; 29:106–25. [PubMed: 7782059]
12. Good M, Honey RC. Dissociable effects of selective lesions to hippocampal subsystems on exploratory behavior, contextual learning, and spatial learning. *Behav Neurosci*. 1997; 111:487–93. [PubMed: 9189263]
13. Holland PC, Bouton ME. Hippocampus and context in classical conditioning. *Curr Opin Neurobiol*. 1999; 9:195–202. [PubMed: 10322181]
14. Jarrell J, Lai EV, Barr R, McMahon A, Belbeck L, O’Connell G. Ovarian toxicity of cyclophosphamide alone and in combination with ovarian irradiation in the rat. *Cancer Res*. 1987; 47:2340–3. [PubMed: 3105875]
15. Jansen C, Miaskowski C, Dodd M, Dowling G, Kramer J. Potential mechanisms for chemotherapy-induced impairments in cognitive function. *Oncol Nurs Forum*. 2005; 32:1151–63. [PubMed: 16270111]
16. Keime-Guibert F, Napolitano M, Delattre JY. Neurological complications of radiotherapy and chemotherapy. *J Neurol*. 1998; 245:695–708. [PubMed: 9808237]

17. Knowlton BJ, Fanselow MS. The hippocampus, consolidation and on-line memory. *Curr Opin Neurobiol.* 1998; 8:293–6. [PubMed: 9635216]
18. Lee GD, Longo DL, Wang Y, Rifkind JM, Abdul-Raman L, Mamczarz JA, et al. Transient improvement in cognitive function and synaptic plasticity in rats following cancer chemotherapy. *Clin Cancer Res.* 2006; 12:198–205. [PubMed: 16397043]
19. Maren S. Neurobiology of Pavlovian fear conditioning. *Ann Rev Neurosci.* 2001; 24:897–931. [PubMed: 11520922]
20. Maren S, Aharonov G, Fanselow MS. Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behav Brain Res.* 1997; 88:261–74. [PubMed: 9404635]
21. McAllister TW, Ahles TA, Saykin AJ, Ferguson RJ, McDonald BC, Lewis LD, et al. Cognitive effects of cytotoxic cancer chemotherapy: predisposing risk factors and potential treatments. *Curr Psychiatry Rep.* 2004; 6:364–71. [PubMed: 15355759]
22. Olin JJ. Cognitive function after systemic therapy for breast cancer. *Oncology.* 2001; 15:613–24. [PubMed: 11396356]
23. Phillips R, LeDoux J. Lesions of the dorsal hippocampal formation interfere with background but not foreground contextual fear conditioning. *Learn Memory.* 1994; 1:34–45.
24. Reiriz AB, Reolon GK, Preissler T, Rosado JO, Henriques JA, Roesler R, et al. Cancer chemotherapy and cognitive function in rodent models: memory impairment induced by cyclophosphamide in mice. *Clin Cancer Res.* 2006; 12:5000. [PubMed: 16914590]
25. Saykin AJ, Ahles TA, McDonald BC. Mechanisms of chemotherapy-induced cognitive disorders: neuropsychological, pathophysiological, and neuroimaging perspectives. *Semin Clin Neuropsychiatry.* 2003; 8:201–16. [PubMed: 14613048]
26. Schagen S, Van Dam F, Muller M, Boogerd W, Lindeboom J, Bruining P. Cognitive deficits after postoperative adjuvant chemotherapy for breast carcinoma. *Cancer.* 1999; 85:640–50. [PubMed: 10091737]
27. Schmidt-Kastner R, Freund TF. Selective vulnerability of the hippocampus in brain ischemia. *Neurosci.* 1991; 40:599–636.
28. Stemmer SM, Stears JC, Burton BS, Jones RB, Simon JH. White matter changes in patients with breast cancer treated with high-dose chemotherapy and autologous bone marrow support. *AJNR Am J Neuroradiol.* 1994; 15:1267–73. [PubMed: 7976937]
29. Stoltenburg-Didinger G. Neuropathology of the hippocampus and its susceptibility to neurotoxic insult. *Neurotoxicol.* 1994; 15:445–50.
30. Troy L, McFarland K, Littman-Power S, Kelly BJ, Walpole ET, Wyld D, et al. Cisplatin-based therapy: a neurological and neuropsychological review. *Psycho-Oncology.* 2000; 9:29–39. [PubMed: 10668057]
31. Tuxen M, Hansen S. Neurotoxicity secondary to antineoplastic drugs. *Cancer Treat Rev.* 1994; 20:191–214. [PubMed: 8156541]
32. van Dam F, Schagen S, Muller M. Impairment of cognitive function in women receiving adjuvant treatment for high risk breast cancer: high dose versus standard dose chemotherapy. *J Natl Cancer Inst.* 1998; 90:210–8. [PubMed: 9462678]
33. Verstappen CC, Heimans JJ, Hoekman K, Postma TJ. Neurotoxic complications of chemotherapy in patients with cancer: clinical signs and optimal management. *Drugs.* 2003; 63:1549–63. [PubMed: 12887262]
34. Wick A, Wick W, Hirrlinger J, Gerhardt E, Dringen R, Dichgans J, et al. Chemotherapy-induced cell death in primary cerebellar granule neurons but not in astrocytes: in vitro paradigm of differential neurotoxicity. *J Neurochem.* 2004; 91:1067–74. [PubMed: 15569250]
35. Winocur G, Vardy J, Binns MA, Kerr L, Tannock I. The effects of anti-cancer drugs, methotrexate and 5-fluorouracil on cognitive function in mice. *Pharm Biochem Behav.* 2006; 85:66–75.

Fig. 1.

Freezing behavior observed during the context test session. Rats treated with cyclophosphamide and doxorubicin exhibited significantly less freezing to the training context than vehicle-treated animals. Data are means $\pm$ S.E.M.

Fig. 2.

Freezing behavior observed during the tone test session. Freezing was comparable for both groups. Data are means $\pm$ S.E.M.