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The Effects of Chemotherapy on Cognitive Function in a Mouse Model: A Prospective Study

Gordon Winocur^{1,2,10}, Mark Henkelman^{3,7}, J. Martin Wojtowicz⁴, Haibo Zhang^{5,8}, Malcolm A. Binns^{1,6}, and Ian F. Tannock⁹

Abstract

Purpose: Clinical studies indicate that up to 70% of patients with cancer who receive chemotherapy experience cognitive impairment. The present study used a prospective longitudinal design to assess short- and long-term effects of commonly used anticancer drugs on cognitive performance in a mouse model.

Experimental Design: Normal mice received three weekly injections of a combination of methotrexate + 5-fluorouracil (CHEMO group) or an equal volume of saline (SAL group). Cognitive tests, measuring different aspects of learning and memory, were administered before treatment, immediately after treatment, and three months later. Structural MRI scanning was conducted at each stage of cognitive testing.

Results: The CHEMO group exhibited deficits on cognitive tasks acquired pretreatment [spatial memory, nonmatching-to-sample (NMTS) learning, and delayed NMTS], as well as impaired new learning on two tasks (conditional associative learning, discrimination learning) introduced posttreatment. Consistent with clinical evidence, cognitive deficits were pronounced on tests that are sensitive to hippocampal and frontal lobe dysfunction, but the CHEMO group's poor performance on the discrimination learning problem suggests that impairment is more widespread than previously thought. Cognitive deficits persisted for at least three months after treatment but some recovery was noted, particularly on tests thought to be under frontal lobe control. The MRI tests did not detect brain changes that could be attributed to treatment.

Conclusions: Chemotherapeutic agents can have adverse effects on information acquired pretreatment as well as new learning and memory and, despite some recovery, impairment is long lasting. *Clin Cancer Res*; 18(11); 3112–21. ©2012 AACR.

Introduction

Clinical studies indicate that up to 70% of patients with cancer who receive adjuvant chemotherapy experience cognitive impairment (1–3). Deficits can be wide ranging but typically include loss of memory and working memory, impaired attentional processes, and poor problem solving—a pattern that suggests dysfunction in hippocampal

and frontal lobe brain areas. The adverse effects of anticancer drugs on cognition have been confirmed in animals and the deficits are often similar to those observed in patients (4–9). The congruence of data from animal models points to the biologic basis of the problem and argues against the notion that such effects are psychosocial in nature, resulting from the challenges of coping with a serious disease and difficult treatment.

While it is accepted that cognitive deficits are a likely secondary effect of chemotherapy, there are unresolved questions related to the nature, severity, and duration of the impairment. For example, are the deficits long lasting? Most studies have examined only short-term effects but the clinical literature suggests that impairment may last for several years posttreatment (10–12; but see ref. 13). If there is recovery over time, do some functions recover faster than others? Is memory for information acquired before chemotherapy affected as much as posttreatment learning and memory?

These and related questions are addressed here in a prospective longitudinal study involving healthy, adult mice administered 3 doses of methotrexate (MTX) and 5-fluorouracil (5-FU) at weekly intervals. These drugs are

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Translational Relevance

Patients with cancer who receive chemotherapy often experience cognitive impairment that adversely impacts quality of life. The problem has assumed greater importance as more people are surviving long-term following treatment. Clinical studies have been useful in identifying chemotherapy-induced cognitive impairment. However, they are limited in their ability to exclude confounding factors, study underlying mechanisms, and identify factors that are relevant to preventing or treating such impairment. The development of animal models has been useful in this regard and the present study, using a prospective longitudinal design, has confirmed that mice injected with a combination of the commonly used anticancer drugs methotrexate + 5-fluorouracil experience widespread cognitive impairment. Deficits were especially pronounced in functions associated with the hippocampus and frontal lobes and, while there was some recovery, there were long-lasting effects. The results provide direction for the development of pharmacologic and behavioral interventions aimed at promoting cognitive recovery.

commonly administered as adjuvant therapy to women with breast cancer, a subset of whom have been found subsequently to exhibit cognitive impairment (12). A comprehensive set of cognitive tests was selected for their sensitivity to disruption of specific cognitive processes that underlie different forms of learning and memory. At all stages of cognitive testing, mice underwent structural neuroimaging in a 7.0 Tesla MRI scanner, and quantitative measures of regional brain volumes were evaluated.

Materials and Methods

Subjects

The study was conducted on female BALB/c mice, 5 months old at the beginning of the experiment, obtained from the Charles River Laboratories. The mice were housed in groups of 3 to 5 and maintained on a reversed 12-hour light/dark cycle (lights on at 1,800 hours and off at 0600 hours). The mice had unlimited access to standard laboratory chow and water and were examined regularly by a veterinarian.

The experimental protocol was approved by the Trent University (Peterborough, ON, Canada) and University of Toronto animal care committees.

General procedures

Mice were assigned randomly to a chemotherapy (CHEMO, $N = 13$) or saline (SAL, $N = 13$) group and underwent structural MRI neuroimaging at the University of Toronto's Mouse Imaging Centre (MICe; Toronto, ON). After transfer to Trent University, the mice were prepared for baseline testing during which 4 learning and memory tests were

administered: spatial memory, nonspatial cued memory, nonmatching-to-sample (NMTS) rule learning, and delayed NMTS (DNMTS). Mice were always tested in groups of 4 or 5.

Beginning 1 week after baseline testing, and each week for 3 consecutive weeks, the CHEMO group received an intraperitoneal injection of MTX (37.5 mg/kg; Wyeth Canada) + 5-FU (50 mg/kg; Mayne Pharma) dissolved in saline, and the SAL group was injected with an equal volume of physiologic saline. The doses for MTX and 5-FU were selected on the basis of dose-response tests for tolerance and toxicity. The dosages selected for the present research were well tolerated and did not affect appetite or activity levels. The only noticeable effect was a small amount of hair loss in a few mice. Higher doses caused significant weight loss and, occasionally, death.

Posttreatment behavioral testing was initiated 1 week after the last injection. The baseline tests were readministered along with 2 new ones: conditional associative learning (CAL) and visual discrimination learning. After post-treatment testing, both groups were transferred to MICe for brain MRI scanning. They were then returned to the Trent animal facility, where they remained in their home cages for 3 months. After this period, long-term follow-up cognitive testing was initiated with the same tests that were administered posttreatment. After follow-up testing, the mice were returned to MICe for a final imaging session and then euthanized.

Over the course of the experiment, there was some attrition in both groups. The numbers of mice that yielded analyzable data at posttreatment and follow-up testing are indicated in the appropriate Results section.

A schematic representation of the study design and timelines is presented in Supplementary Fig. S1.

Cognitive testing

All cognitive tests were administered in a circular pool (130 cm diameter and approximately 30 cm high), located in the centre of a testing room. The pool was filled with opaque water and maintained at 21°C. An inverted flower pot, a few centimeters below the surface, served as a platform on which the mice could climb to escape the water. The water was cleaned after each trial and changed every 2 to 3 days.

The behavioral tasks differed but the objective always was to find a submerged platform to escape the water. For each trial of every test, the mouse was allowed 60 seconds to find and mount the platform. If it failed in that time, it was guided manually to the platform. After 20 seconds on the platform, the mouse was transferred to a holding cage under a heat lamp to await the next trial.

The pool was divided into 6 zones of approximately equal size. Swimming patterns were monitored by an overhead video camera connected to a recorder and data processing system. The system recorded the time required to mount the platform (latency) as well as swimming routes that were used to count errors. An error was recorded each time the mouse entered a zone not containing the platform.

The standard procedure for all cognitive tests was to test the mice in each squad on a given trial and then make the necessary adjustments for the next trial. This resulted in an intertrial interval of about 3 minutes for most tests. The exception was the DNMTS test where, because of the nature of the test, the intertrial interval necessarily varied between 2 and 20 minutes (see later).

For all tests, with the exception of the probe trials of the spatial and cued memory tests, latency and error measures were recorded. If the mouse failed to find the platform within 60 seconds, it received an error score of 15 and a latency score of 60 seconds for that trial. For the probe trials of the spatial and cued memory tests, the time spent in the zone that had contained the platform during the preceding test trials was the only measure.

Spatial memory. The spatial memory test was a variation of the standard Morris water maze (14) used widely as a measure of hippocampal dysfunction.

At baseline, the spatial memory test began with 2 days of orientation training (5 trials/d) in which the mice learned to swim to the platform, which was visible and in a different location on each trial. After 2 days, all mice were reaching the platform within a few seconds. Spatial memory testing began the following day. The platform was now positioned a few centimeters below the surface and always located in the centre of the northeast zone. At the beginning of each trial, the mouse was placed in the water at the edge of the pool, facing the wall, at a different location. The mice never started the trial in the northeast zone, where the platform was located. Each trial continued until the mouse mounted the platform with all 4 paws, or until 60 seconds elapsed. Each mouse received 5 such trials/d for 5 consecutive days. On the sixth day of testing, trials 1 & 2 and 4 & 5 were conducted in the usual manner. On the third trial, which served as a probe trial, the platform was removed and the mice were allowed to swim for 60 seconds before being transferred to the holding cage to await the fourth trial.

Posttreatment and follow-up testing procedures were the same except there was no orientation training, the mice received only 3 days of testing in each session, and the probe trial occurred on trial 3 of day 4.

Cued memory. Performance on the cued memory test is usually affected by extensive brain damage and was included as a measure of the severity of chemotherapy-induced cognitive impairment.

Baseline testing began 2 days after completion of the spatial memory test. For this task, the location of the platform, which varied from trial to trial, was signaled by a gray cylinder (30 cm long \times 3 cm in diameter), suspended 5 cm above the platform. In all other respects, including a probe trial on day 6, testing procedures and scoring were identical to those of the spatial memory test.

Posttreatment and follow-up testing procedures for the cued memory test were the same as baseline, except the mice received only 3 days of testing and the probe trial occurred on trial 3 of day 4.

Nonmatching-to-sample

The NMTS task consisted of a series of paired sample and test trials. In the sample trials, a black or white cylinder (30 cm long \times 3 cm in diameter), suspended 5 cm above the platform, signaled the platform's location. In the subsequent test trial, the platform was relocated and both cylinders were present in different locations. The cylinder that was not present during the preceding sample trial now signaled the platform's location. NMTS and related rule-learning tasks incorporate working memory components and are highly sensitive to frontal lobe dysfunction (15).

At baseline, NMTS testing began 2 days after the cued memory test. For each sample trial, the mouse was placed in the south-east zone of the pool and allowed to swim to the submerged platform under the sample cylinder. The mouse remained on the platform for 20 seconds and was then transferred to the holding cage while the platform was moved and the cylinders put in position for the test trial. The mouse was then placed in the pool and allowed to swim to the submerged platform or until 60 seconds had elapsed. After 20 seconds on the platform the mouse was transferred again to the holding cage, to await the next pair of trials. Ten daily sessions, each consisting of 5 pairs of sample and test trials, were administered.

Posttreatment and follow-up testing procedures were the same except that mice were tested for only 5 days.

Delayed nonmatching-to-sample

The DNMTS test was conducted in the same way as the NMTS test except that a variable interval separated the sample and test trials. As the interval between sample and test trials is increased, greater demands are placed on memory processes and animals with hippocampal impairment perform poorly at longer intervals (16–18). Thus, the NMTS and DNMTS tasks yield dissociable learning and memory functions related respectively to the frontal lobes and hippocampus.

In all sessions, DNMTS testing began the day after completion of NMTS testing and extended over 10 daily sessions. Each session consisted of 4 paired trials, with intervals of 0¹, 60, 120, or 240 seconds between the sample and test trials. The order varied each day according to a random schedule. Scoring for the DNMTS test was identical to that of NMTS testing.

Conditional associative learning

In CAL, the animal must associate one response with a particular stimulus and another response with a different stimulus. This task, widely accepted as a measure of frontal lobe function (15), was administered only in the posttreatment and follow-up sessions to assess the effects of chemotherapy on learning and remembering a new conditional rule.

¹The shortest interval between sample and test trials was the same as in NMTS testing. While designated as 0 seconds, in fact, the interval was about 10 seconds, the time required to prepare for the test trial.

For the CAL task, a cross-maze, constructed of black plastic, was fitted into the pool. Each arm was 27 cm long, extended 10 cm above the water line, and opened into an 11-cm² central area. A black or white cylinder (30 cm long × 3 cm in diameter) suspended 5 cm above the central area signaled the direction of a submerged platform, which was located at the end of one of the arms.

Posttreatment testing began with a 2-day orientation session consisting of 5 trials/d. For each orientation trial, the mouse was placed in the pool at the end of a randomly selected arm. The mouse was allowed to swim to a submerged platform which was located at the end of each arm, except the start arm. There was no cue to direct the animal's directional choice in the central area.

For CAL testing, mice received 8 trials/d in which they were placed individually in the pool at the end of one arm. There was only one submerged platform, located at the end of one of the other arms. In the centre of the maze, the mouse encountered the black cylinder on half the trials, and the white cylinder on the other trials. For half the mice, the black cylinder signaled that the platform was located at the end of the arm to the right; the white cylinder signaled a left turn to find the platform. The reverse was the case for the other mice. The starting position varied from trial to trial so that the location of the submerged platform also varied accordingly. The mouse had 60 seconds to find the platform. Mice were tested for 8 days on the CAL task during posttreatment and follow-up testing.

Brightness discrimination learning

In discrimination learning, the animal learns to discriminate between black and white arms in a T-maze. This task, also administered only at posttreatment and follow-up, measures nonconditional, stimulus–response learning, and is sensitive to impairment in the striatal system (19).

The pool was fitted with a gray, plastic T-maze with walls that extended 10 cm above the surface of the water. The stem of the "T" was 27 cm long; the horizontal arm was 65 cm long with slats along the walls into which black or white panels were inserted.

Posttreatment testing began with a 2-day orientation session consisting of 5 trials/d. For each orientation trial, the mouse was placed in the pool at the end of the stem and allowed to swim to a submerged platform which was located at the end of each goal arm. For these trials, there was no discrete cue to direct the animal.

Discrimination learning began the following day and consisted of 5 trials/d. On each trial, the mouse was placed in the pool at the end of the stem and allowed to swim to the choice point, where it encountered the black- and white-paneled arms. For half of the mice the black arm was positive and for the other half the white arm was positive. The positioning of the panels was determined by a random schedule. A submerged platform was located at the end of the correct arm.

Mice were tested on the discrimination learning task until they reached a criterion of 9 of 10 error-free trials over 2 consecutive days.

MRI

A multichannel 7.0 T, 40 cm diameter bore magnet (Varian Inc.) was used to acquire anatomic images. A custom-built 7-coil array was used to image 7 mice simultaneously in the same gradient set using multiple transmit/receive radiofrequency coils (20). Mice were imaged using standard *in vivo* imaging procedures. A T2-weighted 3-dimensional fast spin-echo sequence was used with image parameters: TR = 2,300 ms, echo train length = 8, TE_{eff} = 36 ms, field-of-view (FOV) = 40 × 24 × 24 mm³, and matrix size = 320 × 192 × 192, giving an image with 125-μm isotropic voxels. The total imaging time was approximately 3 hours.

MRI analysis

An image registration-based approach was used to assess anatomic differences between the brains of CHEMO and SAL groups. Image registration finds a smooth spatial transformation that best aligns one image to another such that corresponding anatomic features are superimposed. The deformation (local expansion, contraction, rotations, and translations) that brings the 2 into alignment thus becomes a summary of how they differ. An automated intensity-based groupwise registration approach (21) was used to align all brains into a common coordinate system, yielding an average image and deformations that relate individual images to this average. The Jacobian determinants of these deformations were extracted, giving a measure of local volume expansion/contraction at every point in the brain. An ANOVA was then computed at every voxel relating this expansion/contraction factor against the water maze training regimen. Multiple comparisons were controlled for using the false discovery rate. In addition, an anatomic atlas labeled with 62 distinct structures (22) was used to compute volumes of each brain structure for each mouse.

Statistical analysis of behavioral results

The measures analyzed for the spatial memory, cued memory, NMTS, DNMTS, CAL, and discrimination learning tests were the average latency (in seconds) and the average number of errors across all trials on each testing day. Only error scores are presented as the latency and error scores yielded the same pattern of results for all tests. Latency data and their analyses are available on request.

For the probe trials on the spatial and cued memory tests, the amount of time spent in the zone where the platform had been located during the learning trials was recorded. The average length of time was analyzed for these probe trials.

ANOVA was used to test differences between groups on behavioral measures. The ANOVA models included group (CHEMO or SAL); days of testing; and the interactions between these 2 factors. Analysis of DNMTS scores included an additional within-subject interval factor (0, 60, 120, and 240 seconds delays). Significant interactions were followed by analysis of simple main effects of treatment group at each interval. Analysis of the discrimination learning data and the probe trial measures did not include the effect of days or interaction in the model.

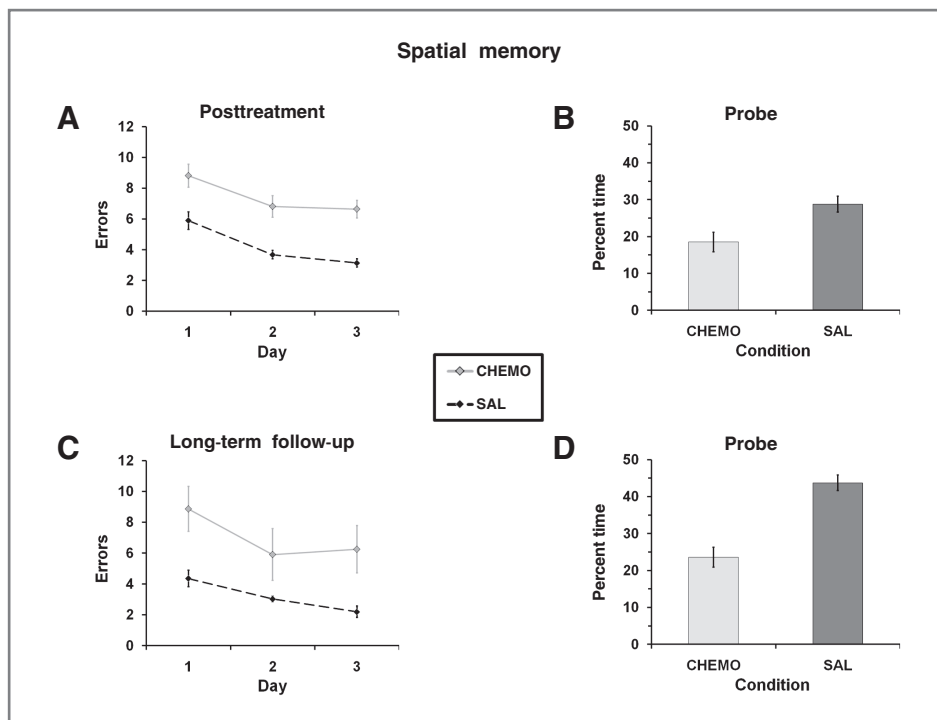


Figure 1. Performance of CHEMO and SAL groups on the spatial memory test at posttreatment (A and B) and follow-up (C and D). Errors (A and C) represent the mean number of errors/d over 3 days of testing. For the probe trials (B and D), the measure is the percentage of time spent in the platform zone. (Error bars, \pm SEM)

To evaluate whether the magnitude of group effects changed between posttreatment and follow-up test sessions to assess relative recovery from chemotherapy over the long-term, repeated measures ANOVA were conducted on the last test days in the 2 sessions.

All tests were conducted at an α level of 5% and statistics were calculated with PASW Statistics version 18.0.0.

Results

Toxicity

Average weight of mice was not significantly different between the CHEMO and SAL groups at baseline or at the end of the experiment ($P > 0.20$ for all comparisons; see Supplementary Table S1). The mice were monitored for possible side effects related to drug treatment (e.g., motor impairment, apathy) but, except for a small amount of hair loss in about 25% of the mice that received chemotherapy, none was detected.

Behavioral results: baseline

For all measures of learning on the spatial memory, cued memory, and NMTS tasks, there was a main effect of days ($P < 0.001$ for all comparisons), indicating that before treatment the CHEMO and SAL groups exhibited significant learning of the respective tasks. On no task was there an effect of group, or a group \times days interaction ($P > 0.40$ for all comparisons). These results confirm that the randomization procedure achieved the desired balance amongst the groups. The baseline results are presented graphically in Supplementary Figs. S2–S5.

Behavioral results: posttreatment

All mice were administered the spatial memory and cued memory tests, but one mouse in the CHEMO group was not available for the NMTS, DNMTS, CAL, and discrimination learning tests.

Spatial memory. The mice improved significantly over the 3 days of testing ($P < 0.001$), but the CHEMO group made more errors (Fig. 1A) in finding the hidden platform than the SAL group ($P < 0.001$). During the probe trial, the CHEMO group spent less time in the platform zone than the SAL group ($P < 0.01$; Fig. 1B).

Cued memory. There were no significant effects related to treatment over the 3 days of testing (Fig. 2A). Similarly, on the probe trial, there was no difference between groups in time spent in the platform zone (Fig. 2B).

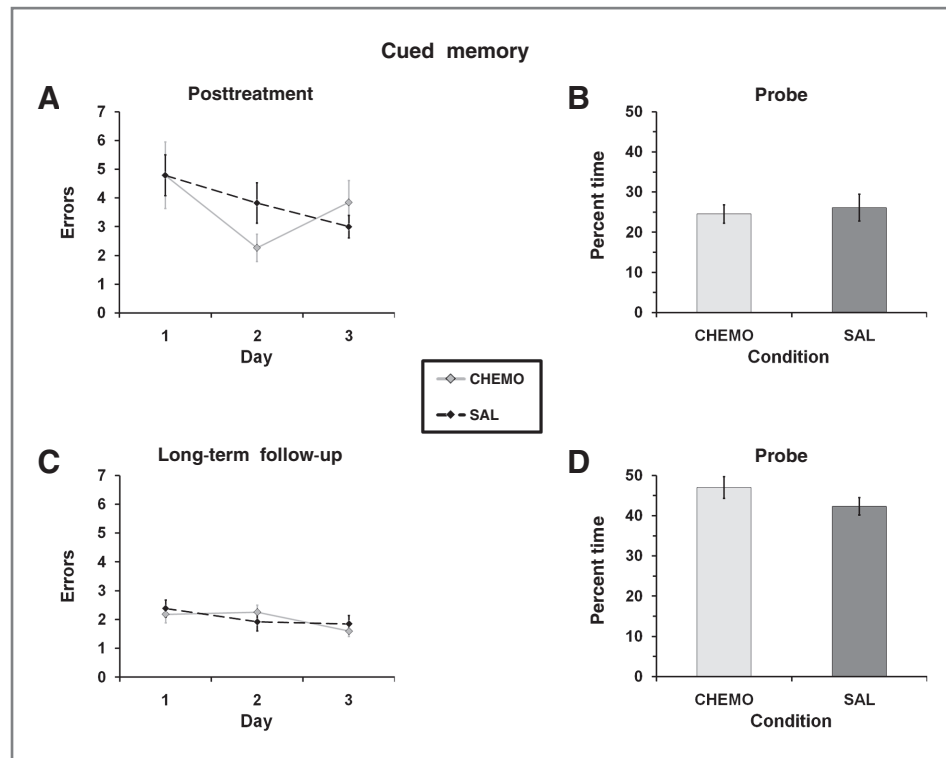
Nonmatching-to-sample

The mice improved on the NMTS task over the 5 post-treatment test days ($P < 0.03$; Fig. 3A), but the CHEMO group made significantly more errors than the SAL group ($P < 0.01$). The treatment \times time interaction was not statistically significant.

Delayed nonmatching-to-sample

For each interval of the DNMTS task, the daily error score per trial for each mouse was averaged over paired successive days. The average scores for both groups are presented in 5 blocks of 2 days in Fig. 4. During posttreatment testing, both groups tended to make more errors as the interval increased, with the effect being more pronounced in the CHEMO group. There was a significant treatment \times interval interaction ($P < 0.002$), as well as a main effect of interval ($P <$

Figure 2. Performance of CHEMO and SAL groups on the cued memory test at posttreatment (A and B) and follow-up (C and D). Errors (A and C) represent the mean number of errors/d over 3 days of testing. For the probe trials (B and D), the measure is the percentage of time spent in the platform zone. Error bars, \pm SEM.



0.001). An examination of simple main effects at each interval revealed that the CHEMO group performed worse than the SAL group at all intervals ($P < 0.01$ for all comparisons), except the 0-second interval.

Conditional associative learning

Over the 8 days of posttreatment CAL testing, the CHEMO group made more errors than the SAL group ($P < 0.001$; Fig. 5A). A significant chemotherapy \times days interaction ($P < 0.01$) confirmed a slower rate of learning in the CHEMO group.

Discrimination learning

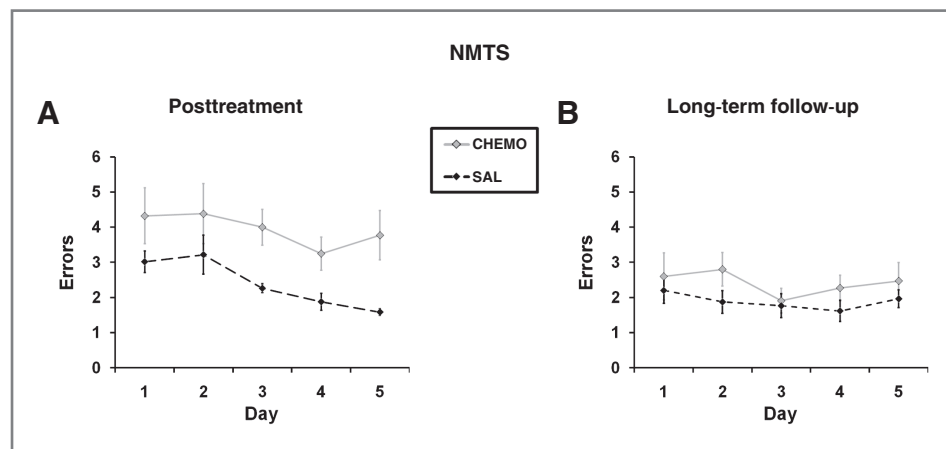
The CHEMO group required significantly more trials than the SAL group to reach criterion ($P < 0.001$; Table 1).

Behavioral results: long-term follow-up

Spatial memory. Over the 3 days of testing, the CHEMO group ($N = 11$) made more errors than the SAL group ($N = 12$; $P < 0.001$; Fig. 1C). The effect of days was not statistically significant ($P = 0.06$). During the probe trial, mice that received chemotherapy spent less time in the platform zone than saline-injected mice ($P < 0.001$; Fig. 1D).

Cued memory. The CHEMO ($N = 11$) and SAL ($N = 11$) groups improved significantly over the 3 days of testing ($P = 0.05$; Fig. 2C) but there were no treatment-related effects. Nor was there a significant group difference in the probe test on day 4 (Fig. 2D).

Figure 3. Performance of CHEMO and SAL groups on the NMTS test at posttreatment (A) and follow-up (B). Errors represent the mean number of errors/trial/d on the test trials over 5 days of testing. Error bars, \pm SEM.



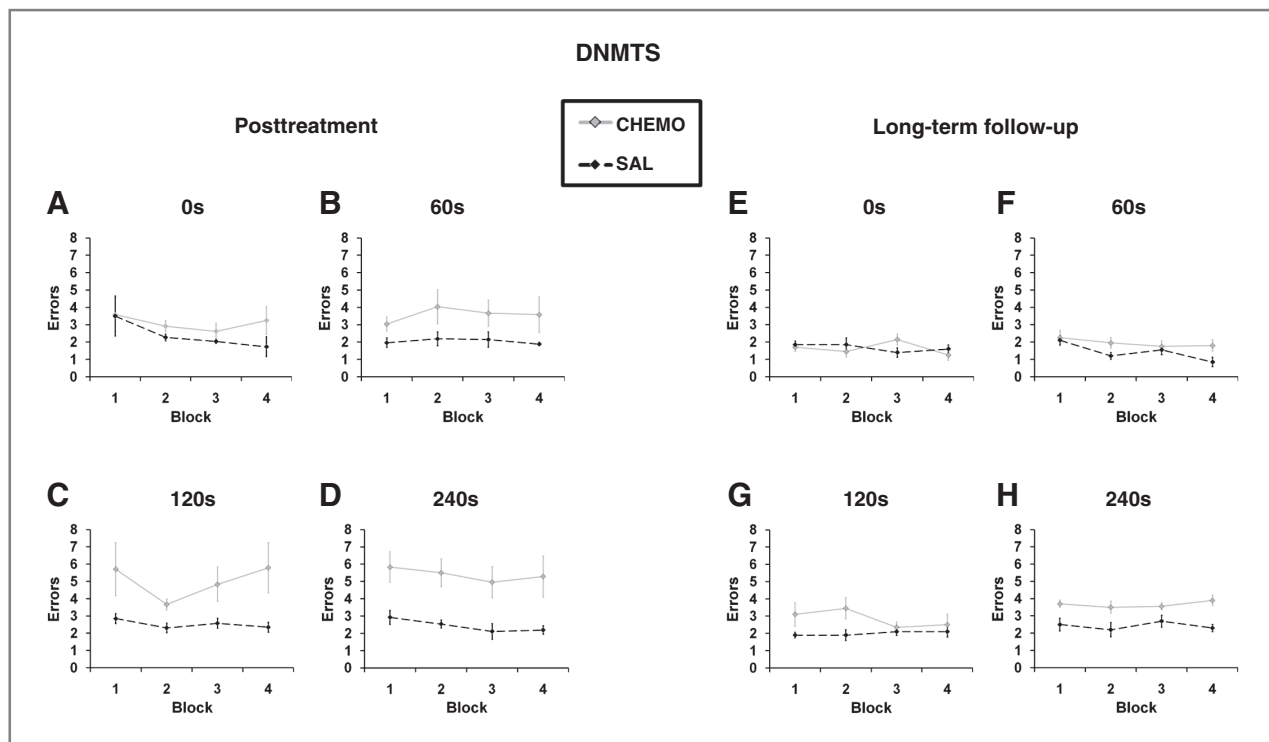


Figure 4. Performance of CHEMO and SAL groups on the test trials at all study test intervals of the DNMTS test at posttreatment (A–D) and follow-up (E–H). Errors represent each group's daily error/trial score averaged over successive days (blocks), presented in 4 blocks of 2 days. Error bars, \pm SEM.

Nonmatching-to-sample

At follow-up on the NMTS test, the CHEMO (N = 10) group continued to perform worse than the SAL (N = 10) group ($P < 0.03$) but the difference between the groups was smaller during follow-up testing than posttreatment (Fig. 3A and B). Repeated measures ANOVA, conducted on the last day of testing in the 2 sessions revealed a significant treatment \times session interaction ($P < 0.01$).

Delayed Nonmatching-to-sample

As in posttreatment testing, at follow-up the CHEMO group (N = 10) made more errors than the SAL group

(N = 10) as the interval increased, with the effect being more pronounced in the CHEMO group (Fig. 4E–H). There was a significant chemotherapy \times interval interaction ($P < 0.002$), as well as a main effect of interval ($P < 0.001$). The CHEMO group performed worse than the SAL group at all intervals ($P < 0.05$ for all comparisons), except the 0-second interval.

Conditional associative learning At follow-up testing on the CAL task (CHEMO, N = 9; SAL, N = 10), there was a main effect of days ($P < 0.001$) but no significant effects related to treatment. There was considerable variability in the early behavior of the CHEMO group. By day 5, this group's performance stabilized and was numerically

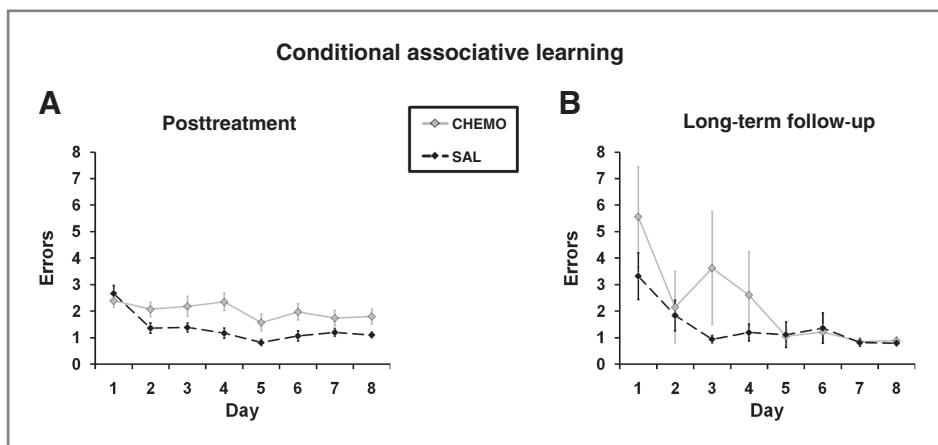


Figure 5. Performance of CHEMO and SAL groups on the CAL test at posttreatment (A) and follow-up (B). Errors represent the mean number of errors/trial/d in over 8 days of testing. Error bars, \pm SEM.

Table 1. The average number of trials required by the CHEMO and SAL groups to reach criterion on the discrimination learning task during posttreatment and long-term follow-up testing

	Discrimination learning			
	Posttreatment		Follow-up	
	SAL (<i>n</i> = 13)	CHEMO (<i>n</i> = 12)	SAL (<i>n</i> = 10)	CHEMO (<i>n</i> = 9)
Mean	21.2	75	12.5	33.3
95% CI	11.9–30.5	48.0–102.0	4.3–20.7	21.8–44.9

Abbreviation: CI, confidence interval.

superior to its performance posttreatment, although a repeated measures ANOVA failed to yield a significant treatment \times sessions interaction ($P < 0.07$; Fig. 5B).

Discrimination learning

Both the CHEMO ($N = 9$) and SAL ($N = 10$) groups improved over their posttreatment performance, but the CHEMO group was still impaired on this task and required significantly more trials than the SAL group to reestablish criterion ($P < 0.001$; Table 1).

Imaging results

MRI was completed successfully in 132 mouse imaging sessions. Two mice died during recovery from anesthesia. All images were successfully registered together into an average.

Compared with the initial scans, there was a small 3% increase in brain volume over the duration of the experiment. There were also significant decreases (22%) in the volume of the frontal lobes as well as in the parietotemporal lobe. There was a smaller increase (10%) in the volume of the hippocampus over the same period. There were, however, no significant differences found in the volume of any brain structure at any point between the CHEMO and SAL groups. Typical averaged images of brains of mice in the CHEMO and SAL groups taken at the completion of the experiment are shown in Supplementary Fig. S6. Changes in volume of several brain regions over the course of the experiment are presented in tabular format in Supplementary Table S2.

Discussion

The present study, which used a prospective longitudinal design, provides further evidence that anticancer drugs can adversely affect cognitive performance. Prospective longitudinal studies of cognitive function following chemotherapy have been conducted in patients with cancer (11, 23–26) and generally the results point to drug-induced impairment. However, these studies have been questioned on several grounds, including sample size, pretreatment cognitive status, and practice effects (see ref. 26). The use of an animal model allows greater control over factors that might confound or undermine the results.

A consistent finding of clinical studies is that chemotherapy disrupts cognition over the short-term, but there is controversy as to the duration of these effects (see ref. 13). Here, we show that chemotherapy-induced cognitive deficits, at least in a mouse model, can be long lasting. Mice that received 3 injections of a combination of MTX + 5-FU continued to exhibit impaired performance 3 months after treatment (see also ref. 6).

In the present study, mice receiving chemotherapy or saline injections were administered a series of cognitive tests that were learned before treatment (spatial memory, cued memory, NMTS, DNMTS), as well as 2 tests for the first time posttreatment (CAL and brightness-discrimination learning). The CHEMO group was impaired on all the tests, except cued memory, which typically is only affected by severe brain damage. This shows that the anticancer drugs disrupted the retrieval of well-established information as well as new learning.

The poor performance of chemotherapy-treated mice on tasks known to be sensitive to hippocampal (spatial memory, DNMTS) and frontal lobe impairment (CAL, NMTS) confirms the susceptibility of these brain regions to the effects of anticancer drugs (see ref. 27). Overall, the pattern of deficit was similar to that observed previously by Winocur and colleagues (4), with one notable exception. In the earlier study (4), performance on the brightness-discrimination task was not affected by MTX + 5-FU treatment. In the present study, despite the fact that the strain of mice and drugs were the same, dosages comparable, and testing procedures identical, the CHEMO group was impaired on this task at short and long intervals following treatment. This is a potentially important finding because discrimination learning entails stimulus response, procedural learning and is known to be affected by damage to the striatal system (19). If confirmed by subsequent investigation, there would be further evidence that the extent of cognitive impairment associated with chemotherapy is greater than previously thought.

It is noteworthy that, in most studies of the effects of MTX or 5-FU on cognitive function, the drugs have been administered individually (refs. 5–8; but see ref. 9). Typically, a single hippocampus-sensitive test of learning and memory is conducted and the consistent finding is drug-induced impairment. Following our approach we showed

that a clinically relevant dose and combination of MTX and 5-FU, despite being well tolerated, produced reliable deficits on a range of cognitive tasks. It remains to be seen whether the individual drugs, administered at various dose levels, would have similar effects.

As indicated earlier, there were long-lasting effects of chemotherapy on cognitive performance. This was especially the case on the hippocampus-sensitive memory tests and the discrimination learning test. There was also some cognitive recovery, notably in terms of frontal lobe function. For example, the posttreatment impairment exhibited by the CHEMO group on the NMTS test reduced substantially at long-term follow-up. Moreover, during follow-up testing there was no evidence of impairment in the CHEMO group at the 0-second interval of the DNMTS test, which essentially replicates the NMTS test. In another example, the CHEMO group was impaired on the CAL task when it was introduced posttreatment. Three months later, after some initial variability in the CHEMO group, there was no longer a statistical difference between the groups. It is possible that a floor effect in the SAL group biased the latter results but, as can be seen in Fig. 5A and B, by day 5 of follow-up testing, there were signs that the CHEMO group's performance had improved over its performance at the end of posttreatment testing.

If frontal lobe functions are indeed amenable to recovery, that could have important implications for treatment (28). The most successful cognitive rehabilitation programs are those that focus on executive function under frontal lobe control and emphasize the effective use of appropriate strategies (29, 30). Moreover, the best results are obtained when patients are sufficiently functional to understand the program's requirements and generalize their training to other tasks and situations. Given the nature of their impairment and their functional status, cancer survivors would appear to be good candidates for such programs, and at least one study has produced encouraging preliminary results: Ferguson and colleagues (31) administered a cognitive training program to 29 patients with breast cancer who had received chemotherapy and, 6 months later, observed significant improvement on several behavioral measures, including neuropsychologic test performance.

In related work (32), we studied the effects of donepezil, a cholinesterase inhibitor used to treat cognitive problems associated with mild cognitive impairment and early Alzheimer disease, on cognitive performance in mice receiving injections of MTX + 5-FU. The results showed that donepezil treatment reduced memory deficits and, to a certain extent, impairment of executive function, in mice administered the anticancer drugs. There is cause for optimism that some combination of cognitive rehabilitation and pharmacotherapy may be effective in relieving cognitive problems resulting from chemotherapy.

It is interesting to note that, in our research, variability in performance of mice in the chemotherapy and control groups was relatively small in comparison with that typically seen in clinical studies. This is a common observation in animal research and can be related to several factors. First,

of course, preclinical studies are conducted on homogeneous samples of animals with similar genetic and experiential histories. In the course of behavioral research, they are well prepared and tested in a rigorously controlled manner on reliable tests designed to measure highly specific functions. This type of control is difficult to exercise with human populations and, indeed excessive control is to be avoided as individual variation is a defining feature of the human condition that must be considered in designing clinical experiments. At the same time, a lesson from the animal work is that it may be possible, through better sampling and methodologic practices, to reduce unwanted sources of variation in clinical investigations. More rigorous efforts along these lines would improve the generalizability of animal-based findings and increase their translational relevance.

Finally, the failure to find changes in the brains of chemotherapy-treated mice, as measured by structural MRI, contrasts with evidence from clinical neuroimaging studies (33, 34), and was unexpected. It is possible that the structural changes in our mouse model were subtle and/or the live imaging techniques were not sufficiently sensitive to detect such changes. Chemotherapy produces numerous physiologic effects on the central nervous system that could account for cognitive deficits. These include oxidative stress, neuroinflammation, and suppression of new cell production (neurogenesis) and nerve growth factors (1, 27, 28). A better understanding of these putative mechanisms is essential for minimizing the impact of anticancer drugs on cognition and for developing biologic treatments.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interests were disclosed.

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