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The effects of the anti-cancer drugs, methotrexate and 5-fluorouracil, on cognitive function in mice

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Abstract

There is evidence that standard-dose chemotherapy may impact cognitive function in cancer patients. The present study evaluated the effects of a combination of two anti-cancer drugs, methotrexate (37.5 mg/kg) and 5-fluorouracil (5FU, 75 mg/kg) on cognitive function in a mouse model. Drug-induced deficits were observed in adult BALB/C mice on tests of spatial memory, non-matching-to-sample (NMTS) learning and in a delayed-NMTS test of non-spatial memory. There were no group differences on tests of cued memory or discrimination learning. Performance-related variables were ruled out as possible explanations of the observed impairments. The impaired performance of the drug group, which was consistent with cognitive deficits observed in human cancer patients treated with similar types of chemotherapy, was attributed to functional changes in specific brain regions, including the frontal lobes and hippocampus.

Keywords: Chemotherapy; Cognitive function; Animal model

Chemotherapy for cancer is associated with side effects that include myelosuppression and its consequent risks of infection, anemia and bleeding, nausea and vomiting, hair loss, gastrointestinal irritation, fatigue and an accelerated menopause in women. There is also growing evidence that standard-dose chemotherapy can impact cognitive function (e.g., Ahles et al., 2002; Bender et al., 2006; Brezden et al., 2000; Donovan et al., 2005; Mar Fan et al., 2005; Meyers, 2000; Schagen et al., 1999; Shilling et al., 2005; Tannock et al., 2004; Tchen et al., 2003; Van Dam et al., 1998; Wefel et al., 2004). Following a recent meta-analysis that included neuropsychological data from 29 studies and 838 patients, Anderson-Hanley et al. (2003) concluded that patients receiving anti-cancer drugs were at significant risk to experience at least mild to moderate cognitive impairment. The most consistent deficits were reported in verbal memory and on tasks that depend on strategic or executive process for successful performance. Similar effects were reported in a second meta-analysis of 16 studies that evaluated the effects of chemotherapy on cognitive function in cancer patients (Jansen et al., 2005). In the clinical neuropsychological literature, these deficits are associated respectively with impaired function in hippocampal (Squire, 1992) and frontal lobe (Stuss and Benson, 1986) regions of the brain.

Investigations of cognitive change following chemotherapy often suffer from limitations that include small samples, less than adequate controls and failure to account for other factors (e.g., disease-related complications, stress) that could affect performance. There are inherent methodological difficulties and ethical concerns associated with conducting this type of research in clinical settings, but until the effects of treatment are separated from potentially confounding variables, conclusions regarding a

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link between chemotherapy and cognitive impairment must be considered tenuous.

One approach in addressing this issue is to investigate the effects of chemotherapy on cognitive performance in animal models. Surprisingly, few studies have taken this approach and, of the few that are available, most focused on 16–17-day-old rats or mice, with mixed behavioural results (Stock et al., 1995; Yadin et al., 1983; Yanovski et al., 1989). Two studies addressed this issue in adult animals. In one, Madhyastha et al. (2002) tested the effects of intracerebroventricular injection of methotrexate in rats and reported drug-induced deficits in avoidance conditioning and locomotor activity. In the other, Lee et al. (2006) compared the effects of two chemotherapeutic agents, cyclophosphamide and 5-fluorouracil (5FU), on cognitive performance in groups of young and aged adults. Somewhat unexpectedly, the drug-treated young rats exhibited transient improvement on tests of spatial memory and complex maze learning. The old rats were tested only on maze learning and there was no effect of treatment in these animals.

In the present study, we compared groups of mice, administered methotrexate and 5-fluorouracil (5FU), a standard form of chemotherapy, or an equal volume of the saline vehicle, on tests of cognitive function. These drugs are used widely in the treatment of human cancer and are components (with cyclophosphamide) of the CMF regimen that has been associated with cognitive changes in women who received adjuvant chemotherapy to prevent recurrence of breast cancer (Brezden et al., 2000; Schagen et al., 1999; Tchen et al., 2003). The behavioral tasks, which tap into a broad range of learning and memory processes, were considered appropriate instruments for an initial assessment of selective effects of chemotherapy on cognitive performance and for investigating the potential for this approach in modeling the cognitive changes associated with anti-cancer drugs in humans.

All tests were conducted in a pool filled with opaque liquid, in which mice were required to find a hidden platform under different conditions. Initially, mice were administered a variation of the standard Morris water maze test of spatial memory (Morris et al., 1982), which is sensitive to hippocampal dysfunction. The mice were also administered a non-spatial test of memory in which a discrete cue signaled the location of the submerged platform. These were followed by a test of non-matching-to-sample (NMTS) rule learning, which is highly sensitive to frontal-lobe dysfunction (Moscovitch and Winocur, 1995; Stuss and Levine, 2002; Winocur and Hasher, 2004), but is not typically affected by damage to the hippocampus (Aggleton et al., 1986; Squire et al., 2004; Zola-Morgan and Squire, 1985). By increasing the interval between sample and test trials, the task puts increased demands on time-dependent, non-spatial memory under hippocampal control (Moscovitch and Winocur, 2002; Winocur, 1992a). The NMTS task, designed in this way, yields dissociable measures of learning and memory functions related respectively to the frontal lobes and hippocampus. A primary question addressed here is whether drug effects are selective to frontal lobe or hippocampal measures, or impact learning and memory performance in a more general way. Finally, the mice received a discrimination learning test in which they had to discriminate between black and white stimuli to find the hidden platform. This task, which is sensitive to effects of lesions to the corpus striatum (McDonald et al., 1999), was included as an additional test of drug effects on cognitive processes that are not typically associated with hippocampal or frontal lobe function.

1. Methods

1.1. Subjects

Twenty-five, female BALB/C mice, obtained from the Charles River Laboratories (Saint-Constant, Québec, Canada), served as subjects. The mice were approximately 2 months old at the time of arrival and they were housed in group cages for an additional 2 months before the experiment began. Throughout the experiment, the mice were housed in plastic shoebox cages (25 × 15 × 10 cm) in groups of 3–5, with free access to both standard lab chow and water. They were maintained on a reversed 12-h light/dark cycle (lights on at 1800 h and off at 0600 h). During this time, their weights were recorded every 3 days.

The experimental protocol was approved by the Trent University Ethics Committee and the mice were examined regularly by a veterinarian. The experimental protocol and all handling procedures conformed to those approved by the Trent University Animal Care Committee and the Canadian Council on Animal Care.

1.2. Apparatus

All testing was conducted in a circular pool (130 cm diameter and approximately 30 cm high), located in the centre of a room (360 cm × 360 cm). The room was illuminated by overhead fluorescent lights. The pool was filled with water rendered opaque by diluted, non-toxic white tempera paint, to a depth of 18 cm, and maintained at room temperature (21 °C). An inverted flower pot (15 cm high) with a white surface (10 cm in diameter), situated a few centimeters below the surface of the water, served as a platform on which the mice could climb to escape the water. A heat lamp was situated near the pool and provided a warm area where mice waited between trials.

Standard laboratory furniture (e.g., testing equipment, a stool, desk, cabinet) was located around the room and several pictures were mounted on the walls. Throughout testing, the water was cleaned after each trial and changed every 2–3 days.

1.3. Drug treatment

A month before behavioural testing, mice were assigned randomly to a drug or control group. Each week for 3 consecutive weeks, mice received an intraperitoneal injection of either (37.5 mg/kg) and 5FU (75 mg/kg) dissolved in saline, or an injection of equal volumes of physiological saline. The dosages were selected on the basis of preliminary work which showed that these doses were tolerated with minimal weight loss; higher doses caused weight loss or death of animals. Methotrexate was obtained from Wyeth Canada, Thornhill, Ontario and 5FU was obtained from Mayne Pharma, Kirkland, Québec.

Subsequent behavioural testing was conducted by a different experimenter who was blind to the treatment history.

1.4. Spatial memory

One week after the final injection, testing began on the spatial memory test. Initially, mice received 2 days of orientation to the pool. On each day, the mice received five trials in which they were placed in the pool and allowed to swim to and climb upon the platform, which, for these trials, was visible a few cm above the surface of the liquid. The location in which the mice were placed in the pool and the location of the platform were varied from trial to trial. A trial continued until the mouse mounted the platform with all four paws, or until 120 s elapsed. The mouse was allowed to remain on the platform for 20 s; if it failed to find the platform in the allotted time, it was placed on the platform for 20 s. The mouse was then removed and placed in a clean cage under the heat lamp to await the next trial. The mice were run in squads of 4–5, allowing for an interval of 2–3 min between trials. Latency to reach the platform was recorded.

Spatial memory testing began on day 3. For this test, the pool was divided into four quadrants—NE, NW, SE and SW, with the platform, now below the surface of the water and always located in the centre of the NE quadrant. For each trial, the mouse was placed in the water at the edge of the pool, facing the wall, at one of the four cardinal compass points—N, E, S and W. The starting positions were determined by a semi-random sequence, with the condition that each starting point was used at least once each day. Trial administration was identical to that followed in training, with each trial continuing until the mouse mounted the platform with all four paws, or until 120 s elapsed. As before, the mouse was allowed to remain on the platform for 20 s; if it failed to find the platform in the allotted time, it was placed on the platform for 20 s. The mouse was then removed and placed in a clean cage under the heat lamp to await the next trial. Each mouse received 5 trials/day for 5 consecutive days, following this procedure. On day 6, the first two trials 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 s. Trials 4 and 5 followed the usual procedure with the submerged platform returned to its location.

Two response measures were recorded for each trial of days 1–5—latency and errors. The latency was the time required to climb onto the platform, measured from when the mouse was placed in the water. If the mouse failed to find the platform within 120 s, it was given a score of 120 for that trial. An error was counted each time the mouse entered a quadrant not containing the platform, or when the mouse left the NE quadrant without successfully mounting the platform. During the probe trial of day 6, the interest was in how much time the mouse spent in the quadrant that normally contained the platform, and so only this measure was recorded on this trial.

1.5. Cued memory

Testing on the cued memory task began 48 h after completion of the spatial memory test. For this task, a grey cylinder (30 cm long × 3 cm in diameter), suspended 5 cm over the submerged platform, served as a cue for the platform's location. The quadrant in which the platform and cue were located was changed for each trial according to a semi-random schedule, with the qualifier that they would not be located in the same quadrant for more than two consecutive trials. The position of the cylinder was controlled manually by the experimenter through a system of pulleys, weights and wires that ran inconspicuously outside the perimeter of the pool and along the ceiling.

For each trial, the mouse was placed in the water as described for the test of spatial memory. The mouse was never placed in the quadrant containing the submerged 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.

1.6. Non-matching to sample (NMTS) learning

The NMTS task consisted of a series of paired sample and test trials. The stimuli for the sample and test trials were black and white cylinders (30 cm long × 3 cm in diameter), suspended 5 cm above the surface of the water. The position of the cylinders was controlled as described for the non-spatial memory test. As in the previous tasks, the water maze was divided into four equal quadrants with invisible boundaries between them.

At the beginning of each sample trial, the black or white cylinder was suspended 5 cm above the submerged platform. During the subsequent test trial, both cylinders were present but the cylinder that was not present during the preceding sample trial was suspended over the platform and cued its location. Thus, if on a given sample trial, the black cylinder cued the platform, then, on the succeeding test trial, the white cylinder cued the platform. The location of the cylinder and platform varied between sample trials. The black and white cylinders were selected as sample stimuli for each pair of trials according to a semi-random schedule that ensured that each cylinder was the sample stimulus for 50% of the trials. For each test trial, the platform was moved to another quadrant with the non-sample cylinder located directly above it. The sample stimulus was also moved to a different quadrant. The quadrant that contained the submerged platform was changed after each sample and test trial, according to a random schedule, in order to eliminate the use of spatial cues. All quadrants were used equally for locating cues in the sample and test trials and, within the quadrants, the platform was positioned randomly.

Testing on the NMTS task began 2 days after the completion of the cued memory test. At the beginning of each sample trial, the mouse was placed in the pool at a variable location, facing the wall of the pool, and allowed to swim to the submerged platform under the sample cylinder. The mouse remained on the platform for 20 s. The mouse was then removed and placed in a clean cage under the heat lamp while the platform was moved and the cylinders put in position for the test trial. The organization of the cylinders and platform took about 10 sec. The mouse was then placed in the pool and allowed to swim to the submerged platform or until 120 s had elapsed. In either case, the mouse was allowed 20 s on the platform before being returned to a holding cage under a heat lamp, to await the next pair of trials. The mice were tested in squads of 4–5, which

allowed for an interval of 4–5 min between each pair of trials. Ten daily sessions, each consisting of five pairs of sample and test trials, were administered.

Latency and error scores for each sample and test trial were recorded. Latency represents the time taken to swim to and climb on to the platform after the mouse was placed in the water. An error was recorded whenever a mouse's entire body entered an incorrect quadrant.

1.7. Delayed-NMTS (DNMTS)

The day after the completion of NMTS training, mice were administered ten additional daily sessions. Each session consisted of four paired trials, with delays of 0, 60, 120 or 240 s between the sample and test trials. (The delays do not include the 10 s required for repositioning the cylinders and platform.) The order of the delays varied each day according to a random schedule. During DNMTS testing, the interval between successive pairs of trials varied, ranging from approximately 2 min when the sample-trial delay was 0 s, to 20–25 min when the sample-trial delay was 240 s. In all other respects, the testing procedure and scoring for delayed testing was identical to that of NMTS learning.

1.8. Black–white discrimination learning

For this task, the pool was fitted with a T-maze that consisted of three arms (55 cm × 15 cm) that extended from a central area (15 cm^2) at 90° angles. The walls of each arm consisted of interchangeable black or white panels. The submerged platform was located a few centimeters below the water line at the end of the panel designated as the positive arm. The walls on the sides of each arm extended 12 cm above the water line.

Testing in the black–white discrimination task began 2 days after completion of the DNMTS test. For half the mice, the black arm was positive and, for the other half, the white arm was positive. The stem was positioned at one of the four cardinal compass points and changed on every trial according to a semi-random schedule, with the condition that each starting point was used at least once each day. Similarly, the position of the black and white panels on each trial was determined by a semi-random schedule with the condition that the panels were not configured in the same way for more than two consecutive trials.

At the beginning of each trial, the mouse was placed in the stem at the edge of the pool and allowed up to 120 s to find and mount the submerged platform. The mouse was then allowed to remain on the platform for 20 s. If it failed to find the platform within 120 s, it was picked up and placed on the platform for 20 s. At the end of the trial, the mouse was placed in a clean cage under the heat lamp to await the next trial. Each mouse received 5 trials/day until a criterion of 80% errorless trials over 2 consecutive days was reached.

For each trial, the latency to mount the submerged platform and the number of errors were recorded. An error was scored each time the mouse's entire body entered the incorrect arm and when the mouse left the correct arm after having entered it.

1.9. Statistical analysis

Analysis of variance was used to test for differences between drug and control treatment groups on the weight records and behavioural measures. For the spatial memory, cued memory, NMTS and DNMTS tests, we recorded the length of time taken to find the platform and the number of errors committed in the process. The dependent behavioural measures for these tests were the average length of time and the average number of errors across all of the trials on each testing day. On the two probe trials for the spatial and cued memory tests, during which the platform was removed from the pool, the dependent measure was the average length of time spent in the quadrant where the platform normally would have been. For the black–white discrimination task, the dependent measures were the total number of errors made and the number of trials required to reach criterion on each testing day. The models for all analyses of variance contained a between-subject treatment group factor (methotrexate + 5FU or saline), a within-subject testing day factor (days 1 to 5), and the interaction between these two factors—probe trial measures did not include a day effect or interaction in the model. Significant treatment × day interactions

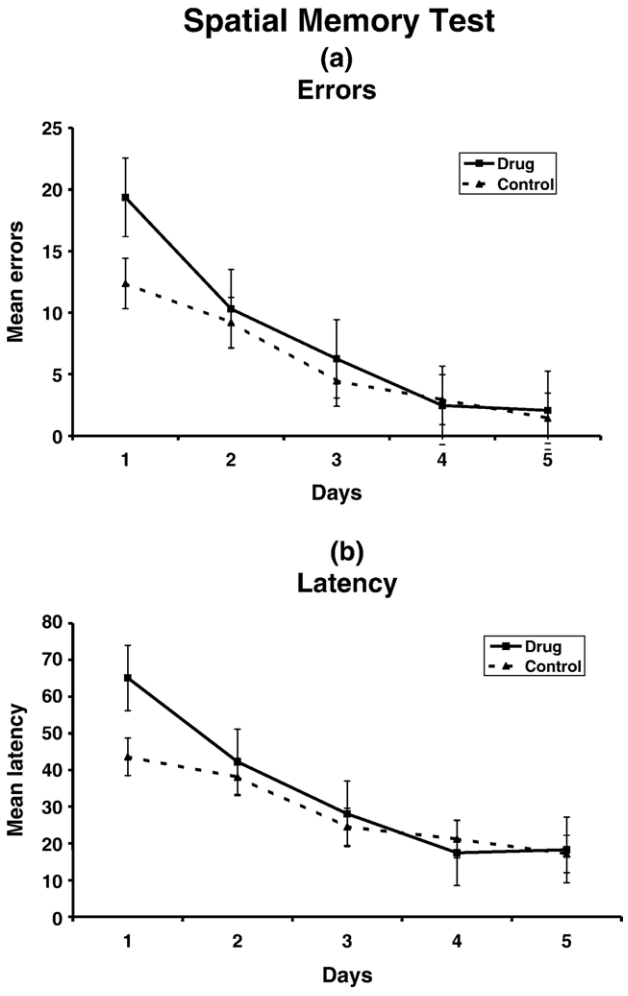

Fig. 1. Performance of drug and control groups on the spatial memory test. (a) Mean number of errors per day; (b) mean latency (s) per day to find the submerged platform. Error bars denote the standard error of the mean.

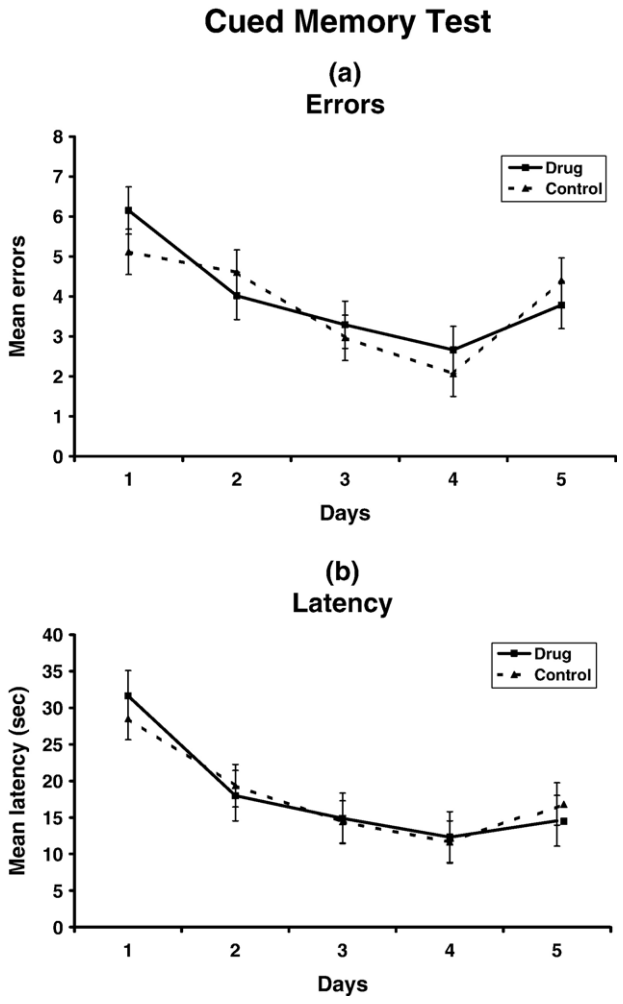

Fig. 2. Performance of drug and control groups on the cued memory test. (a) Mean number of errors per day; (b) mean latency (s) per day to find the submerged platform. Error bars denote the standard error of the mean.

would be followed by a priori simple main effect analyses of treatment group at each day using the appropriate pooled error term as described in Kirk (1968). Analyses of DNMTS measures included an additional within-subject delay factor (0-, 60-, 120- and 240-s delays). Significant treatment × delay interaction would be followed by analysis of simple main effect of treatment group at each delay. Tests of significance were performed at an alpha level of 5% and statistics were calculated using SPSS version 12.0.1.

2. Results

2.1. Weight records/toxicity effects of chemotherapy

At the beginning of the study, the average weights for the drug-treated and control mice were 19.3 g [S.E.M.=0.29] and 18.8 g [S.E.M.=0.26], respectively. At the end of the study, the averages were 20.5 g [S.E.M.=0.37] and 20.8 g [S.E.M.=0.21] for the drug-treated and control groups, respectively. ANOVA, conducted on the weights confirmed a significant effect of time, $F_{1,23}=82.86$, $p<0.0001$, that represented weight gain in the two groups. There were no differences between the groups in weight gain, nor was there a group × time interaction ($p>0.35$ for both comparisons).

The mice were monitored for possible side effects related to drug treatment (e.g., motor impairment, apathy), but, except for loss of a small amount of facial hair in a handful of mice, none was detected.

2.2. Spatial memory

All mice quickly learned to find the visible platform during preliminary training and, after 2 days, were reaching the platform within a few seconds. There was no difference between groups in terms of latency to find the platform.

As can be seen in Fig. 1, on day 1 of spatial memory testing, drug-treated mice made more errors (Fig. 1a) and took more time (Fig. 1b) than the control group to find the hidden platform. By day 2, however, there were no longer differences between the groups. These observations were confirmed by ANOVA, which yielded statistically significant treatment × day interactions on both measures (errors: $F_{4,92}=4.53$, $p=0.002$; latency: $F_{4,92}=4.79$, $p=0.001$). The main effect of treatment was significant on the error

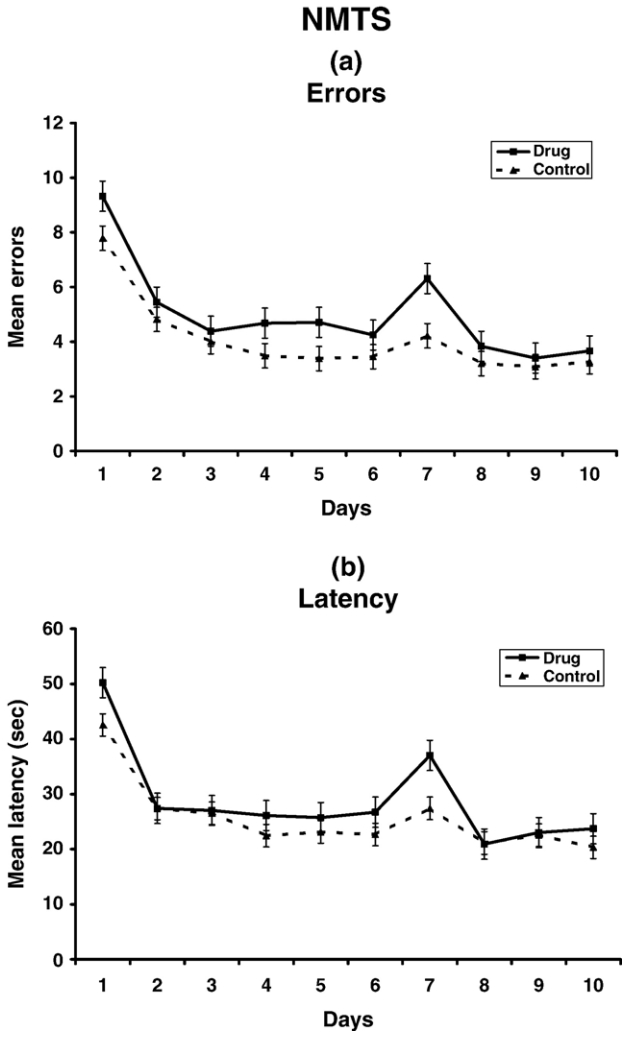

Fig. 3. Performance of drug and control groups on NMTS test over 10 test days. (a) Mean number of errors per day; (b) mean latency (s) per day to find the submerged platform. Error bars denote the standard error of the mean.

measure ($F_{1,23}=8.31, p=0.008$) and was marginally significant on the latency measure ($F_{1,23}=3.79$, $p=0.06$). The ANOVA also revealed a highly significant main effect of day on both measures (errors: $F_{4,92}=72.52$, $p<0.0001$; latency: $F_{4,92}=50.49$, $p<0.0001$). The significant interaction was due to the poorer performance of the drug group on day 1 as revealed by analysis of the simple effects of group at each day: day 1, $F_{1,115}=24.70$, $p<0.0001$; day 2, $F_{1,115}=0.6$, $p=0.43$; day 3, $F_{1,115}=1.6$, $p=0.20$; day 4, $F_{1,115}=0.10$, $p=0.74$; day 5, $F_{1,115}=0.20$, $p=0.66$. For the probe trial of day 6, analysis of the time spent in the area associated with the submerged platform revealed no significant difference between the groups (average time: drug: 34.0 s [S.E.M.=5.19]; control: 36.6 s [S.E.M.=4.10]; $t<1$).

Considering only the first trial of day 1 testing, comparisons were performed to determine if the group differences were apparent from the very beginning of testing or if they developed over the subsequent trials. These analyses revealed that, even on trial 1, the drug group had longer latencies (75.2 s [S.E.M.=10.27] vs. 44.3 s [S.E.M.=9.53; $t_{23}=2.21$, $p=0.04$) and made more errors (22.2 s [S.E.M.=3.68] vs. 11.4 s [S.E.M.=2.58]; $t_{23}=2.39$, $p=0.03$) than the control group.

2.3. Cued memory

The results for the cued memory test are presented in Fig. 2. There was no significant treatment × day interaction or main effect of treatment or day on the error (Fig. 2a) or latency (Fig. 2b) measure during the 5 days of testing ($F<1$ for all comparisons). Nor was there a group difference in time spent in the area associated with the submerged platform during the probe trial of day 6, $t<1$.

2.4. NMTS

There were no performance differences between the drug and control groups on the sample trials of the NMTS and DNMTS tasks, in terms of latency and errors made in finding the platform

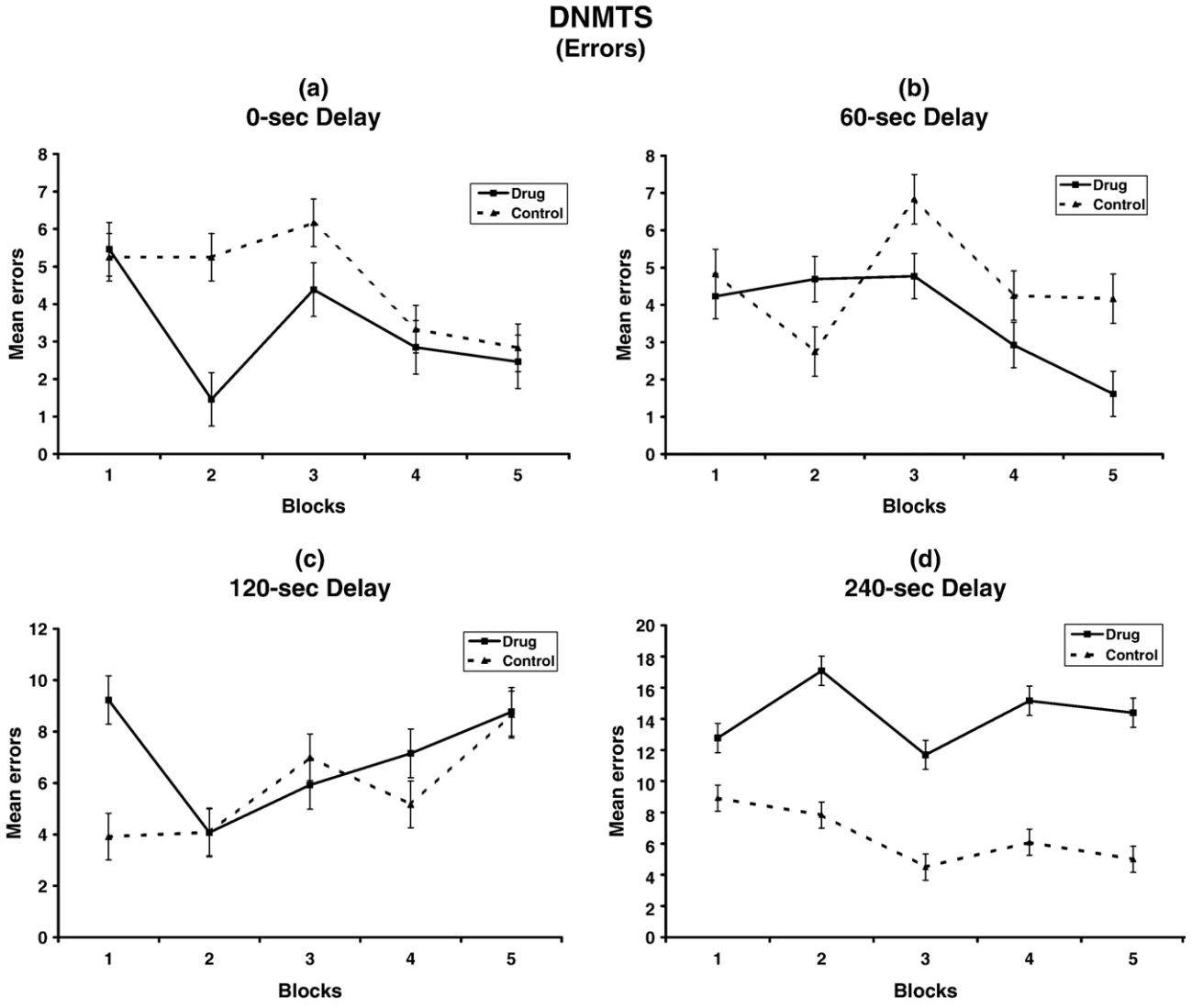

Fig. 4. Mean number of errors made by drug and control groups at all delays of the DNMTS test. Data are presented over 5 blocks of 2 days each. Error bars denote the standard error of the mean.

($p>0.05$ for all comparisons). Consequently, data are presented only for the test trials.

The performance of drug and control groups in the NMTS learning task is presented in Fig. 3a and b. The drug group consistently made more errors than the control group over the entire test period. Although differences due to treatment on the error measure were small in absolute terms, they were consistent across days and statistically significant, $F_{1,23}=8.84$, $p=0.007$. The main effect of repeated testing days was also significant, $F_{9,207}=16.89$, $p<0.0001$, but the treatment × day interaction was not, $F<1$. On the latency measure, only the effect of day was significant, $F_{9,207}=19.48$, $p<0.0001$.

2.5. DNMTS

The mean number of errors at each delay and the mean latency to find the hidden platform in the test trials were averaged over the 10 test days and are presented in blocks of 2 days each in Figs. 4 and 5, respectively. Overall ANOVA revealed significant treatment × delay interaction on the latency, $F_{3,92}=17.64$, $p<0.0001$, and error, $F_{3,92}=21.46$, $p<0.0001$, scores. To further investigate the simple effect of treatment, a separate ANOVA was conducted at each delay. These analyses revealed a significant deficit in the drug group only at the longest (240-s) delay. At that delay, the drug group made more errors, $F_{1,92}=67.43$, $p<0.0001$, and took longer, $F_{1,92}=31.09$, $p<0.0001$, to find the platform than the control group. There was no effect of treatment, repeated test days, or a treatment × day interaction on either measure ($p>0.18$ for all comparisons) at any of the other delays. This includes the 0-delay condition, where there appears to be a facilitatory effect of the drug (see Figs. 4a and 5a). In this condition, the treatment × day interactions (errors: $F_{4,368}=0.63$, $p=0.64$; latency: $F_{4,368}=1.22$, $p=0.30$), the main effects of treatment (errors: $F_{1,92}=1.74$, $p=0.19$; latency: $F_{1,92}=2.08$, $p=0.15$) and test days (errors: $F_{4,368}=1.62$, $p=0.17$; latency: $F_{4,368}=1.68$, $p=0.15$) were all statistically non-significant.

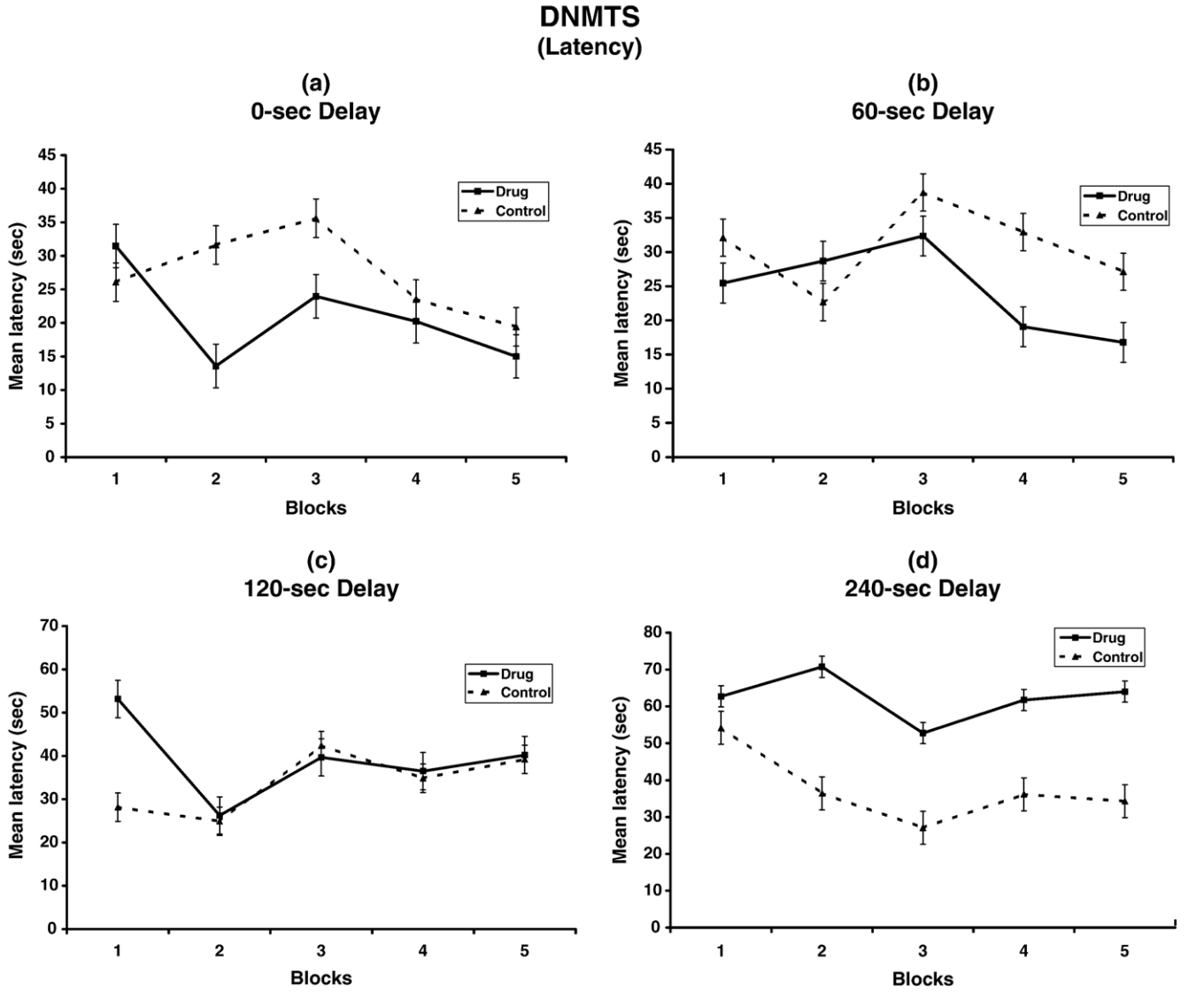

Fig. 5. Mean latency (s) for drug and control groups to reach the submerged platform at all delays of the DNMTS test. Data are presented over 5 blocks of 2 days each. Errors bars denote the standard error of the mean.

Table 1
The average number of trials and errors to criterion by drug and saline control groups on the black–white discrimination task

	Drug		Control	
	Trials	Errors	Trials	Errors
Mean	18.85	20.54	14.17	14.92
S.D.	7.68	10.09	8.48	6.82

2.6. Black–white discrimination learning

The average number of trials required and errors made by both groups before reaching criterion on the discrimination task are presented in Table 1. Although the drug group had higher scores on both measures, the differences, analyzed by *t*-tests, were not statistically significant ($p > 0.05$ for all comparisons).

3. Discussion

The results demonstrate, in an animal model, learning and memory impairment following treatment with methotrexate and 5FU, a drug combination that has been used widely in the treatment of human breast cancer. Drug-induced deficits were observed on the Morris water maze test of spatial memory, in conditional rule learning on the NMTS test, and at the longest delay on the DNMTS test of non-spatial memory. There were no differences between drug and control groups on tests of cued memory or simultaneous, black–white discrimination learning.

The pattern of normal and impaired cognitive function reflected in these results provides insight into brain mechanisms that were affected by the drugs. The behavioral tasks were selected for this study because they assess various aspects of learning and memory which can be dissociated and linked to different brain regions. For example, spatial memory, as measured in the Morris water maze test, is a form of reference memory that depends on the functional integrity of the hippocampus (Morris et al., 1982). The ability to learn the NMTS rule, presumably because of the inherent strategic and working memory components, is identified with frontal lobe function (Moscovitch and Winocur, 1995, 2002). When the interval between sample and test trials is substantially lengthened, thereby challenging time-dependent memory processes, successful performance requires additional support of the hippocampus (Winocur, 1992a,b; Zola-Morgan and Squire, 1985). The consistent impairment of the drug group on independent measures of frontal lobe and hippocampal function provides evidence of the susceptibility of these brain regions to this form of chemotherapy. The drug group was not impaired on the cued memory test or in discrimination learning, tasks that are not affected by selective damage to the frontal lobes or hippocampus, but which appear to depend on the caudate nucleus and related striatal structures (McDonald et al., 1999). This indicates that the adverse effects of this treatment regimen of methotrexate and 5FU probably do not extend to all regions of the brain.

Some of the drug effects on learning and memory, while statistically reliable, were relatively small, especially when compared with changes that result from lesions to the appropriate brain regions. For example, the spatial deficit, observed in the Morris water maze test, was transitory and occurred only on day 1 of testing. Similarly, in the test of NMTS learning, while the drug group consistently made more errors and took longer to find the platform over the 10-day test period, the differences were quite small and, by day 8, were no longer present. The exception to this pattern was the large deficit exhibited by the drug group in the 240-s delay condition of the DNMTS task. The magnitude of the latter effect may reflect the combined demands of having to integrate the strategic and long-tem memory requirements of the task in this particular condition.

It is important to consider whether the behavioural differences between mice treated with methotrexate and 5FU and the controls are due to drug-induced effects on performance-related variables, rather than on cognitive processes. This possibility seems especially relevant to the drug-induced deficit in spatial memory, which was only observed on day 1 of testing. In particular, since this was the first test administered, it is possible that drug-treated mice engaged in more thigmotaxic swimming, which could account for group differences on this task. This is unlikely for the following reasons. First, as indicated in Methods, before testing began, all mice had received 10 trials of preliminary training in the water pool to acquaint themselves with the general task of finding a platform in the water. A few mice did engage in some thigmotaxic swimming but, by the end of training, this was minimal. Indeed, absence of thigmotaxis was a criterion for moving forward to the spatial memory test. Second, on the fourth or fifth trial of each day's testing on the spatial memory test, we made tracings of each mouse's swim pattern. The tracings were drawn on a representative template of the water pool, so that, at least for one trial each day, it was possible to estimate the percent of the swimming path spent along the walls, which provided a measure of thigmotaxis. On day 1, where there were group differences on errors and latency, very little thigmotaxic swimming was seen and drug and control groups did not differ on this measure, $t_{23} = 0.58$, $p = 0.56$.[1]

It is also possible that group differences in the spatial memory test could be the result of hyper-arousal or hyper-activity in the mice administered methotrexate and 5FU. On other measures for which the drug-treated mice were impaired (NMTS learning; DNMTS—240-s delay), they might have been less motivated or suffered a loss of motor function that affected their ability to perform the tasks. Several lines of evidence argue against these interpretations. The drug-treated and control mice gained equal amounts of weight over the course of the study and, apart from minor hair loss in a few animals, there was no sign of drug-related toxicity. The mice were examined regularly by a veterinarian who reported that all mice were healthy throughout the study. The mice were extremely motivated to find the platform in water maze tasks and, indeed, across the various tests, there were only a few trials in which the 120-s time limit to find the platform was reached, and the groups did not differ in this regard. Most importantly, drug effects were not observed on all measures, as

[1] The tracings also provided an estimate of distance traveled by each rat. On the traced trials. the drug-treated and control rats swam an average of 673.5 cm and 342.7 cm, respectively. This difference was statistically significant, $t_{23} = 3.62$, $p = 0.001$.

would be expected if the drugs affected arousal or activity levels, motivation, the ability of mice to navigate through the pool or related performance variables. As indicated above, drug effects were observed on the spatial memory test, NMTS learning and at the longest delay of the DNMTS task. There were no group differences on the cued memory and discrimination learning tasks. Nor were there differences between drug and control groups in their ability to find the platform in the sample trials of the NMTS and DNMTS tests. Thus, the most likely explanation of the poorer performance of mice treated with drugs is that they exhibited genuine cognitive impairment related to functional changes in specific regions of the brain.

Since the tests were administered in the same order to all mice, the question arises as to whether, in some way, an order effect influenced the outcome. For example, it is conceivable that there might have been a build-up of interference with repeated testing that differentially affected the drug group. However, if that were the case, minimally, one would expect the treatment effect to increase progressively over testing. There was no indication of this and, in fact, on the last test administered, simultaneous discrimination learning, there was no difference between drug and control groups. Alternatively, the control group might have benefited more than the drug group from repeated testing and, with experience, developed more efficient strategies. Once again, that would have led to differences on the discrimination test and none were observed. Overall, the evidence overwhelmingly points to drug-induced deficits in memory and executive cognitive processes, related to hippocampal and frontal-lobe dysfunction, that are expressed in tasks that are dependent on these processes.

It is noteworthy that behavioural testing was initiated 2 weeks after the last treatment and completed within 4 to 5 weeks. Thus, in effect, we assessed relatively short-term effects of the drugs. This is important because it raises the possibility that, at longer treatment-test intervals, there might have been some recovery of cognitive function. Along these lines, Lee et al. (2006) reported that rats treated with chemotherapeutic drugs temporarily performed better on cognitive tests than control rats, after initially exhibiting impaired long term potentiation in the hippocampus. In that study, improved cognitive performance was observed 7 to 9 weeks after the last treatment. By 42 weeks following chemotherapy, there were no longer performance differences between drug and control groups. These findings contrast with those of other reports (e.g., Madhyastha et al., 2002; Yadin et al., 1983; Yanovski et al., 1989) and the present one, although all studies had important differences related to species, age, drugs and dosages, cognitive tests and testing schedules. Clearly, a conclusive statement regarding the nature and pattern of cognitive changes following treatment with different chemotherapeutic agents must await systematic investigation that takes into account these variables.

The impaired performance of the drug group in the present study is generally consistent with reports of cognitive impairment in cancer patients treated with chemotherapy. Importantly, the present results show that anti-cancer drugs can adversely affect brain function, apart from the potentially confounding physical and psychological changes that result from the disease. The results also parallel the human data in two other respects. First, as in many clinical reports (Anderson-Hanley et al., 2003; Jansen et al., 2005), the behavioural pattern of the drug-treated mice indicates primary deficits in strategic/executive and memory functions, thought to be controlled by frontal lobe and hippocampal brain regions respectively. More work is needed to establish the full extent of cognitive change following chemotherapy, but our initial findings suggest that the effects do not extend to all brain regions involved in cognition. Second, when cognitive loss is reported in chemotherapy-treated cancer patients, the effects are typically mild to moderate in severity, and while they may impact day-to-day functioning, are not necessarily apparent. As noted above, the learning and memory deficits of the drug-treated mice in the present study, while statistically reliable, for the most part, were relatively small. This is encouraging because, in addition to cognitive-enhancing drugs that target hippocampally controlled memory function (Grundman and Thal, 2000), there are rehabilitation programs designed to promote recovery of executive and memory functions, and they tend to be most effective in patients whose deficits are mild to moderate (Anderson et al., 2003). Thus, there may be treatment options for cancer patients who require chemotherapy but whose quality of life has been diminished by the effects of the drugs on cognitive function.

A number of questions arise from the present results as well as those that emanate from neuropsychological investigations of chemotherapy-treated cancer patients. The present study shows that the combination of methotrexate and 5FU can adversely affect cognitive function. It remains to be determined whether other forms of chemotherapy have similar effects and whether chemotherapy interacts with the disease state to exacerbate the degree of cognitive impairment. As well, there has been limited study of cognitive change in patients with cancer that does not involve the central nervous system (Tucha et al., 2000; Tuma and DeAngelis, 2000). These are important questions that have considerable clinical and practical relevance, and the present results show that animal models can be useful in addressing them.

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