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Preserved learning and memory following 5-fluorouracil and cyclophosphamide treatment in rats

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ABSTRACT

Some patients experience enduring cognitive impairment after cancer treatment, a condition termed "chemofog". Animal models allow assessment of chemotherapy effects on learning and memory per se, independent of changes due to cancer itself or associated health consequences such as depression. The present study examined the long-term learning and memory effects of a chemotherapy cocktail used widely in the treatment of breast cancer, consisting of 5-fluorouracil (5FU) and cyclophosphamide (CYP). Eighty 5-month old male F344 rats received contextual and cued fear conditioning before treatment with saline, or a low or high dose drug cocktail (50 mg/kg CYP and 75 mg/kg 5FU, or 75 mg/kg CYP and 120 mg/kg 5FU, i.p., respectively) every 30 days for 2 months. After a 2-month, no-drug recovery, both long-term retention and new task acquisition in the water maze and 14-unit T-maze were assessed. Neither dose of the CYP/5FU cocktail impaired retrograde fear memory despite marked toxicity documented by enduring weight loss and 50% mortality at the higher dose. Acquisition in the water maze and Stone maze was also normal relative to controls in rats treated with CYP/5FU. The results contribute to a growing literature suggesting that learning and memory mediated by the hippocampus can be relatively resistant to chemotherapy. Future investigation may need to focus on assessments of processing speed, executive function and attention, and the possible interactive contribution of cancer itself and aging to the post-treatment development of cognitive impairment.

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1. Introduction

It is well documented that a percentage of cancer patients experience cognitive impairment associated with treatment, often referred to as "chemofog" (Anderson-Hanley et al., 2003; Falleti et al., 2005; Jansen et al., 2005; Stewart et al., 2006). The nature, degree and duration of impairment are influenced by a number of factors including the type of cancer and treatment, the age, sex, health and hormonal status of patients, and the co-occurrence of depression, anxiety and fatigue. Although early investigations suggested that a large percentage of patients present with a diffuse pattern of deficits, recent studies have generally reported narrower effects restricted to processing speed, attention, mental flexibility and working memory, against a background of preserved autobiographical and remote memory (Anderson-Hanley et al., 2003; Hermelink et al., 2008; Hermelink et al., 2007; Jenkins et al., 2006; Scherwath et al., 2006; Wefel et al., 2004). In addition, a growing clinical literature has noted that, after acute chemotherapy effects subside, long-term cognitive impairment is observed selectively among patients who experience problems during or soon after treatment (Fan et al., 2005; Hermelink et al., 2007; Jenkins et al., 2006; Schagen et al., 2002). Findings of this sort have focused attention on the endurance characteristics of impairment as a key feature of the chemofog profile, raising the possibility that the initial response to therapy and cancer itself may play a significant role. Although the incidence, magnitude and duration of deficits vary across reports, these effects significantly compromise the quality of life for some patients (Stanton, 2006), and a better understanding of the underlying mechanisms is critical toward the development of effective preventions or interventions (Tannock et al., 2004; Vardy et al., 2008).

Animal models offer several advantages for assessing the cognitive consequences of chemotherapy per se, independent of confounding factors. Beyond eliminating the influences of depression, cancer itself, and other comorbidities, studies in rodent models substantially benefit from: a) the decreased cognitive variability and consistent treatment response that can be expected in genetically identical rats and mice relative to people, b) the exacting experimental control that can be exercised over the type, duration and dosing of chemotherapy treatment, and c) the ability to comprehensively document treatment effects in specimens typically unavailable in human clinical investigation. In addition, by using standardized behavioral assessments adopted

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from research on the neurobiology of learning and memory, the specific profile of impairment induced by chemotherapy can provide clues about the underlying brain regions involved (Squire, 2004).

To date, the majority of relevant animal studies have examined the acquisition and retention of new information shortly after the completion of chemotherapy, typically within a few weeks of treatment (Gandal et al., 2008; Li et al., 2008; Macleod et al., 2007; Madhyastha et al., 2002; Mustafa et al., 2008; Seigers et al., 2008; Winocur et al., 2006; for exceptions see Fardell et al., 2010; Mondie et al., 2010). While appropriate for documenting the acute effects of anticancer drugs, such designs are limited on other counts. As noted elsewhere (Marin et al., 2009), neuropsychological performance assessed during or immediately following treatment can be influenced by illness and general malaise, independent of direct effects on the neural systems responsible for cognitive function. Although experimental and statistical approaches can partly control for the contribution of fatigue, anxiety, depression and other potential confounders, studies in animal models provide a window on effects attributable to anticancer agents, per se, independent of the psychological impact of receiving a cancer diagnosis, and the potential role of cancer itself. Given that the clinical profile of chemofog involves persistent cognitive dysfunction, the endurance of impairment that results from anticancer treatment is an important and tractable target for investigation in animal models.

Based on this background, the present experiments tested if the combined administration of two drugs commonly used to treat breast cancer, 5-fluorouracil (5FU) and cyclophosphamide (CYP), results in enduring learning and memory deficits in a well controlled rat model. A cocktail of 5FU and CYP was provided monthly for 3 months, more closely mimicking the temporally distributed nature of clinical treatment regimens compared with the weekly treatments typically used in rodent studies. A battery of behavioral tasks was administered, providing a window on the functional integrity of multiple brain systems known to support learning and memory, and examining both acquisition and retention under a range of motivational and motor demands. To assess the influence of treatment on remote memory, established before the onset of treatment, we tested long-term fear memory for contextual fear conditioning, a capacity independent of the hippocampus (Frankland et al., 2006) but that requires the anterior cingulate cortex (Frankland et al., 2004). In order to document the endurance characteristics of potential deficits in remote memory outside the window when impairment might result from treatment-related illness, retention was tested 2 months after the last drug administration. To determine the influence of the treatment on new learning, next we trained rats on a standard Morris water maze protocol, documenting the effects of prior treatment on spatial learning and memory that critically requires hippocampal integrity (Morris et al., 1982). A final assessment tested acquisition of a complex navigational route using a 14-unit T-maze that depends on hippocampal and striatal processing (Jucker et al., 1990). By comparison with earlier animal studies that have evaluated performance shortly after drug administration (Foley et al., 2008; Konat et al., 2008b; Liedke et al., 2009; Macleod et al., 2007; Reiriz et al., 2006; Seigers et al., 2008; Winocur et al., 2006; for exceptions see Fardell et al., 2010; Mondie et al., 2010) our design provided a window on a key feature in the clinical presentation of chemofog, i.e., the persistence of cognitive impairment long after the acute toxic effects of therapy have resolved.

2. Material and methods

2.1. Subjects

Eighty, 5-month-old male Fischer-344 (F344) rats from Harlan Laboratories, Inc., (Indianapolis, IN) were pair-housed in a vivarium at the Gerontology Research Center of the National Institute on Aging, Baltimore, MD. Rats were maintained on a 12:12 h light–dark cycle at 21 ± 3 °C, 60 ± 10° humidity with free access to food (NIH-07) and chlorinated water. At the start of the study, animals were implanted with a subcutaneous transponder that provided identification and body temperature information (Bio Medic Data Systems, Inc., Seaford, DE). Due to weight loss and dental problems associated with drug administration, treated rats received supplemental food prepared daily. Normal rodent chow was ground to a powder, and mixed with water and sugar. All treatments and testing occurred during the light cycle, and were in accordance to NIA Animal Care and Use Committee guidelines.

2.2. Drug doses

Low (LD, n = 28) and high dose (HD, n = 28) groups received, respectively, a cocktail of 50 mg/kg CYP plus 75 mg/kg 5FU, and 75 mg/kg CYP plus 120 mg/kg 5FU in 0.9% sterile saline, solubilized at 60 °C (all drugs from Sigma-Aldrich, St. Louis MO). The control group (n = 24) received saline. Treatment was administered 4 h after the start of the light cycle. Rats caged together received the same treatment. Drugs were administered i.p. at a concentration such that the injection volume was 0.2 to 0.8 ml. The selection of doses was based on pilot experiments where higher doses resulted in more than 50% mortality (data not shown). These experiments also showed that mortality was reduced when the dose was titrated from low to high. Accordingly, the first treatment in the HD group was the same as in group LD, and only the second and third injections were provided at the higher dose.

2.3. Treatment groups and test schedule

In overview, all rats were trained in the fear conditioning task on day zero, and the next day received the first of three drug injections. The second treatment was on day 30, and the third on day 60. After a subsequent 60-day recovery period, retention of fear conditioning was assessed on day 120. A subset of the animals was then subsequently tested on the 14-unit T and water maze tasks. Fig. 1 presents a schematic of the experimental design.

At the start of the experiment, rats (n = 80) were randomly assigned to one of the three conditions (saline control, Con, n = 24; LD, n = 28; or HD, n = 28), and trained in the fear conditioning task. Roughly half the animals in each group served as no shock controls to determine if baseline motor movement was affected by either the 5-month retention period or drug treatment (i.e., declining motor activity associated with the long retention interval or the drug itself would preclude a straightforward interpretation of the results; Control-no shock, n = 12; Control-shock, n = 12; LD-no shock, n = 12; LD-shock, n = 16; HD-no shock, n = 12; HD-shock, n = 16).

The first drug treatment was delivered the day after fear conditioning. One week after the first and third treatments, and 1 week before testing fear conditioning retention, a subgroup of rats (Con, n = 8; LD, n = 8; HD, n = 8) was lightly anesthetized with ketamine (75 mg/kg, i.p.) and whole blood was collected via the tail vein in capillary tubes for hematocrit determination. Samples were spun in a microhematocrit microcapillary centrifuge for 5 min at 12,000 rpm and the percentage of packed cells was estimated. Three days after each blood draw, all rats in the blood analysis group plus a few additional subjects (Con, n = 13; LD, n = 12; HD, n = 13) were assessed for body fat composition and temperature. Specifically, lean and fat mass were measured simultaneous using a Minispec LF90

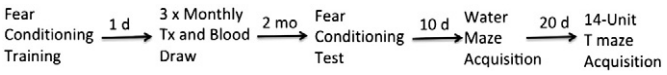

Fig. 1. Experiment time-line.

NMR system, designed to analyze whole body composition in live, unanesthetized rodents (Bruker Optics, Billerica, MA). Two months after the third and last drug injection (Day 120), all rats were assessed for contextual and cue fear memory. Ten days later (Day 130) subjects in the Con (n = 10), LD (n = 10) and HD (n = 9) groups started 5 days of water maze testing. On Days 150–170 a subgroup of rats tested in the water maze received training in the 14-unit T-maze (Con, n = 7; HD, n = 7).

2.4. Behavioral testing

2.4.1. Fear conditioning

2.4.1.1. Apparatus. Two identical conditioning chambers (30×24× 21 cm, MED Associates, St. Albans VT) constructed of aluminum (two sidewalls) and Plexiglass (rear wall, ceiling, and hinged fronted door) were situated within a sound-attenuating cubicle. Behavior in the test chamber was recorded using a digital video system at 15 frames per second, and freezing was analyzed with commercially available software (Video Freeze, MED Associates, Albans VT). The conditioned stimulus (CS) was an 85 dB 2800 Hz 20-second tone delivered though speakers located inside the chamber, and the unconditioned stimulus (US) was a scrambled foot shock at 0.75 mA presented during the last 2 s of the CS. Freezing was defined as the absence of movement except for respiration. For a linear analysis, the software was used to establish a motion detection sensitivity threshold (100 a.u. for the training and context test sessions, 60 a.u. for the cue test session) that was held constant across rats. The minimum freeze duration was 0.73 s.

2.4.1.2. Procedure. Rats were placed in the test chamber for 3 min before three CS/US pairings were delivered, separated by 1 min. Contextual fear memory was tested 120 days later. Retention was assessed by returning rats to the test chamber and recording freezing over 3 min. No shock was given during the context test. Cue fear memory was assessed the next day by measuring the response to CS presentations delivered in a modified chamber intended to be distinct from the initial training context. A plastic insert was used to alter the geometry of the conditioning chamber, the grid floor was replaced with woodchip bedding, and visual patterns on the apparatus wall were changed. Rats were placed in the test chamber 3 min before 3 CS presentations separated by 1 min.

2.4.2. Water maze

2.4.2.1. Apparatus. Extra-maze visual cues were hung from a curtain located around a 1.75-meter diameter tank. The water was 21 ± 1 °C and made opaque by adding white non-toxic paint. A 10-cm diameter escape platform was located 2 cm below the surface of the water and a video-based tracking system (HVS Image, UK) recorded searching during training and probe trials (location, distance and latency). The software system digitally mapped swim paths throughout 4 equal quadrants of the maze, with one centered on the escape location.

2.4.2.2. Hidden platform procedure. Rats were provided 4 trials a day, for 4 consecutive days with an intertrial interval of 10 min. At the start of each trial the rat was placed in the tank, facing the wall, at one of four equally spaced pseudorandom start locations around the perimeter of the apparatus. Trials ended when the rat located the platform or after 60 s. Rats that failed to locate the platform within the 60 second cutoff were gently guided to the goal. Animals remained on the platform for 10 s at the end of each trial. The 5th daily test session consisted of a 60 second probe trial during which the platform was removed and quadrant search time and platform crossings were measured.

2.4.2.3. Visual cued procedure. The same day, after the probe test, rats were tested for their ability to locate a visible platform. Across trials the platform was randomly located in one of the four quadrants and rats entered the water from pseudorandom start locations. Testing comprised 6 trials with a 30 second intertrial interval. Latency to find the platform was recorded.

2.4.3. 14-unit T-maze

2.4.3.1. Apparatus. Both the straight runway used for one-way active avoidance pretraining and the 14-unit T-maze have been described in detail previously (Ingram, 1988). Briefly, the clear Plexiglas runway was 2 m long with a stainless steel grid floor wired to deliver a scrambled foot shock (0.08 mA) when required. Rats entered and exited the runway via identical start/goal boxes positioned at either end. The 14-unit T-maze was of the same general construction as the straight runway but included a series of 14 right/left choice points. The maze was divided into 5 sections by guillotine doors that could be closed remotely to keep rats from backtracking. Movement was monitored using infrared photocells located throughout the maze and this information was transferred to a computer for subsequent analysis. Errors were defined as deviations from the correct path to the goal.

2.4.3.2. Procedure. During pretraining conducted 1 day before testing in the 14-unit T-maze, rats were required to traverse the straight runway and enter the goal box within 10 s to avoid foot shock. Successful completion of pretraining was defined as 13 or more avoidances during 15 consecutive trials, to a maximum of 30 trials. Subsequent training in the 14-unit T-maze was conducted as described previously (Ingram, 1988). Briefly, successful maze navigation required a fixed sequence of left/right turns at 14 choice points between the start and goal box. Rats were permitted 10 s to negotiate each of the five maze segments to avoid a foot shock. The shock was terminated when rats reached the next segment of the apparatus. Rats were given a single session of 15 massed trials with a 2 minute intertrial interval. Data were grouped into three blocks of five trials for analysis.

2.5. Statistical analysis

Body weight, temperature, lean/fat mass ratio and hematocrit levels at specific time points were analyzed by one-way ANOVA, with significant observations followed by Dunnett's post hoc comparison test. Retention in the fear-conditioning task and probe trial performance in the water maze were analyzed via one-way ANOVA. Water and 14-unit T-maze acquisition were analyzed by repeated measures ANOVA, with trials or trial-block as a within-subject variable.

3. Results

3.1. CYP/5FU caused weight loss and high mortality

Fourteen of 28 rats in the HD group (50%), and 5 of 28 in group LD (18%) died before behavioral testing. Mortality generally occurred within a week of drug administration and no rats died after the start of behavioral assessment. The following distribution of animals completed the fear conditioning component of the study: saline Con-no shock, n = 12; saline Con-shock, n = 12; LD-no shock, n = 8; LD-shock, n = 15; HD-no shock, n = 5; HD-shock, n = 9. All rats that received drug lost weight and stopped eating normal rat chow 4–6 days after each treatment. Since many developed dental problems (loose teeth, mouth sores), for the duration of the experiment, drug-treated rats received supplemental chow, ground to a powder and mixed with water and sugar. Despite this intervention, treated rats remained underweight relative to controls throughout the study (Fig. 2). Measured 8 weeks (day 120) after the last treatment, a one-way ANOVA for body weight revealed a significant effect of drug administration, ($F_{2,51} = 38.18$,

$p<0.0001$). Both the LD and HD groups weighed less than controls ($p<0.001$ for both comparisons) but did not differ from each other.

Body temperature, hematocrit levels and body composition were measured taken 6–8 days after the first and third treatments, and again following 8 weeks of recovery. Body temperature and the ratio of lean to fat body mass did not differ among groups at any time point (Body temperature group effect ($F_{2,27}=1.9$, $p>0.10$); lean/fat ratio group effect ($F_{2,27}=2.3$, $p>0.10$)). As shown in Table 1, hematocrit levels were significantly reduced in LD and HD groups relative to controls 1 week after the first ($F_{2,17}=7.2$, $p<0.01$) and third ($F_{2,15}=12.1$, $p<0.01$) treatments, but recovered by the start of post-treatment behavioral testing. Posthoc analyses confirmed that hematocrit levels for both the LD and HD groups were significantly lower than controls ($p<0.01$ for all comparisons), but that levels were similar across drug dosages ($p>0.05$). No significant treatment effect was observed at the end of the 8-week recovery period ($F_{2,11}=2.3$, $p>0.05$).

Table 1
Mean hematocrit levels (SEM) following CYP/5FU treatment.

	Group		
Time point	Control	LD	HD
TX1	42.0 (1.8)	37.0 (0.7)*	36.8 (1.2)*
TX3	43.6 (1.5)	40.6 (0.8)*	37.4 (0.3)*
Recovery	44.3 (1.8)	45.3 (2.9)	46.8 (0.9)

* $p<0.05$ relative control.

3.2. Remote memory for contextual and cue fear conditioning is spared following CYP/5FU treatment

During the three-minute baseline period (before the first tone delivery) on the training day (day zero), all rats actively explored the test chamber. Freezing levels averaged less than 5% with no differences across groups (data not shown). After the 120-day delay, rats were returned to the same chamber to assess contextual fear memory. Freezing in all groups was greater during testing than training, documenting significant long-term retention. As shown in Fig. 3A, there were no statistically significant differences in the percent time freezing among the control, LD and HD rats that did not receive the shock during training ($F_{2,27}=0.57$, $p>0.05$). Importantly, percent freezing in rats that received foot shock was greater than among the no-shock controls ($p<0.01$ for all comparisons), confirming that the response reflected contextual fear memory rather than freezing associated with drug treatment or the 120-day retention period itself. Results of a one-way ANOVA additionally documented that retention failed to differ across treatment groups ($F_{2,34}=1.5$, $p>0.05$; Fig. 3A). It is worth noting however, that to directly compare activity in the context test with baseline activity during training, only initial (3 min) freezing behavior was assessed. Possible effects of chemotherapy on the magnitude of freezing over time, or on extinction are not detectable in the present experiment.

Nonetheless, these findings indicate that all three groups receiving shock exhibited robust long-term contextual fear memory, and moreover, that chemotherapy had no detectable effect on retention in this experimental setting.

In order to examine retention of the tone/shock association itself, independent of contextual cues, the next day rats were placed in modified test chamber intended to comprise a novel environment. Similar to results for the context test, all groups displayed higher levels of freezing during the 3-minute baseline (before the onset of the first tone) than during the same interval of the training trial, but there were no differences among the saline, LD and HD groups within the shock/no-shock training conditions (data not shown). The basis of the non-specific increase in freezing in no-shock controls is unclear but may reflect a neophobic response to the repeated injections, generalization between the different environmental contexts, or other experimental procedures that all subjects experienced. To normalize for individual variability in baseline activity in the new context, a difference score was calculated by subtracting the average percent time freezing during the pre-tone baseline on the cue test trial from percent freezing across the three CS presentations. (Averaging across the three tone presentations was done after statistical analysis showed equivalent freezing at each tone by all tone/shock groups.) By this measure, rats exposed to tone/shock pairings during training froze significantly more than tone alone controls ($p<0.05$ for all comparisons). Freezing increased for all tone/shock groups from the first presentation (65–80%), to the second and third presentations (85–95%). Against this background of robust long-term memory, the magnitude of retention was statistically equivalent across the Con, LD and HD groups ($F_{2,34}=0.9$, $p>0.1$; Fig. 2B). These data show that remote memory for cued fear conditioning remains intact following low or high dose administration of a CYP/5FU cocktail. To directly

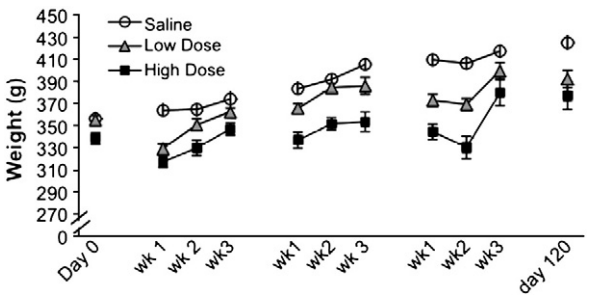

Fig. 2. Mean body weight (g) of saline controls and rats treated with three injections of low dose or high dose of the CYP/5FU cocktail. Weights are presented before the first treatment (day 0), for the 3 weeks following each treatment, and after a 2-month recovery from the last treatment at the time of fear conditioning testing (day 120).

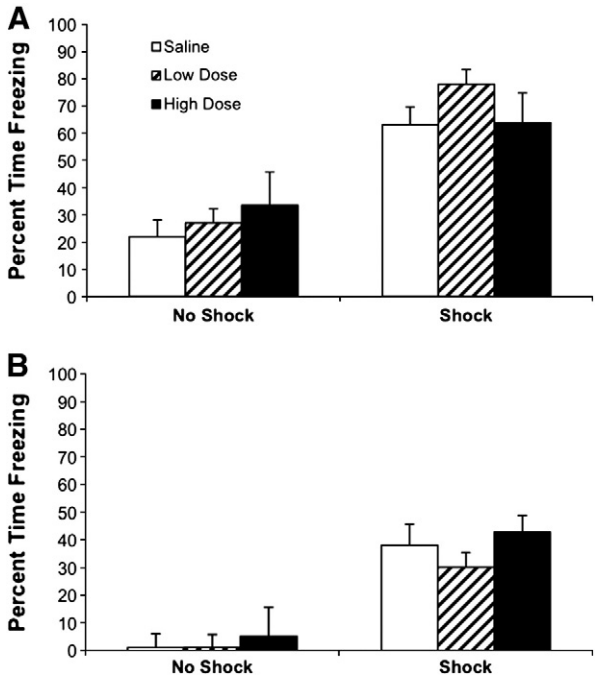

Fig. 3. Freezing behavior observed in the contextual (A) and cued (B) fear memory test session. A) Rats receiving the foot shock during training exhibited good memory for context as reflected by their increased freezing compared to no shock controls. CYP/5FU cocktail did not impair performance. B) Difference in percent time freezing at baseline relative to percent time freezing across the three tone presentations during the cue test trial (0 = no retention). CYP/5FU cocktail did not impair performance.

match the training condition, freezing behavior was assessed for three tone presentations and freezing at each tone presentation statistically equivalent among the groups

3.3. Morris water maze performance is spared following CYP/5FU treatment

On day 130, a subset of rats from the saline Con (n = 10), LD (n = 10) and HD (n = 9) groups was assessed for spatial learning and memory using a 5-day water maze protocol. Subjects included in this assessment had all received foot shock during fear conditioning. Fig. 4A shows mean latency to locate the submerged platform across the 4 daily test sessions (4 trials/day). Performance improved in all groups across days, confirming a significant learning effect (repeated measures ANOVA main effect of day, $F_{3,78} = 17.8$, $p<0.01$), but there was no effect of treatment ($F_{2,26} = 1.05$, $p>0.1$) or day × treatment interaction ($F_{6,78} = 0.31$, $p>0.5$). Swim speed was not different among the groups ($F_{2,26} = 2.00$, $p>0.1$) and the distance to the goal platform mirrored the latency data. That is, a repeated measures ANOVA showed a main effect of day ($F_{3,78} = 18.2$, $p<0.01$), but there was no effect of treatment ($F_{2,26} = 1.10$, $p>0.1$) or day × treatment interaction ($F_{6,78} = 0.36$, $p>0.5$). These findings indicate that water maze acquisition was intact following long-term recovery from CYP/5FU administration. A probe test in which the platform was removed from the apparatus was provided approximately 24 h after the last training trial, and the percentage of time rats searched in the vicinity of the previous escape location was taken as a measure of spatial memory. As shown in Fig. 4B, all groups spent significantly more time in the target quadrant compared to the directly opposite quadrant of the apparatus, and more than would be expected by chance (i.e., 25%, $p<0.05$ for all comparisons). In addition, the average distance rats searched from the goal location during the probe test was not different among the groups ($F_{2,26} = 1.90$, $p>0.1$) Against this background of significant retention, there was no indication of a treatment effect at either dose of CYP/5FU. Consistent with the conclusion that chemotherapy fails to yield enduring impairment in water maze performance, the drug and control groups also scored comparably on a one-session, cued platform variant of testing (Group effect ($F_{2,26} = 2.17$, $p>0.1$)).

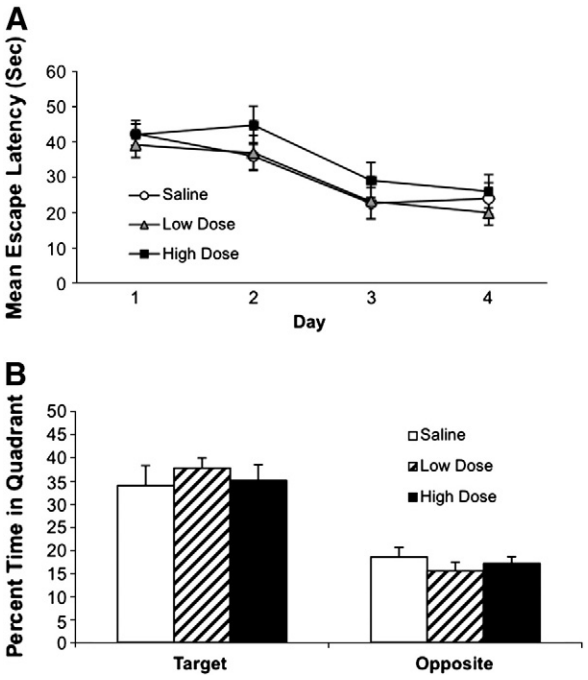

Fig. 4. Acquisition of a hidden escape location in the water maze (A) and spatial bias during probe testing (B). A) All groups showed significant learning, reflected by decreasing escape latencies across days. B) Rats exhibited robust memory for the goal location on probe tests, reflected as strong search bias for the quadrant of the maze that contained the escape platform during training. CYP/5FU cocktail did not impair performance.

3.4. 14-unit T-maze acquisition is spared following CYP/5FU treatment

On Days 150–170 a subgroup of rats tested in the water maze received training on the 14-unit T-maze. Given the lack of impairment revealed by other assessments, only saline controls (Con-shock, n = 7) and HD rats (HD-shock, n = 7) were tested. There was no difference between groups at any point in training. As shown in Fig. 5, both groups learned rapidly (repeated measures ANOVA main effect of trial block, $F_{2,24} = 83.4$, $p<0.01$), and there was no effect of treatment ($F_{1,12} = 1.3$, $p>0.1$) or block × treatment interaction ($F_{2,24} = 0.6$, $p>0.5$).

4. Conclusions

The present experiments used a battery of multiple learning and memory tasks in rats to document the long-term cognitive effects of two drugs used widely in the treatment of breast and other cancers. Low and high dose cocktails of cyclophosphamide (CYP) and 5-fluorouracil (5FU) both induced marked toxicity including transient hematocrit reduction, persistent loss of body weight, and substantial mortality. Against this background of robust physiological effects, remote memory of conditioned fear, acquisition of a complex response sequence, and spatial learning and memory assessed in the Morris water maze were all entirely normal in drug-treated subjects relative to controls. The results indicate that learning and memory requiring the hippocampus and other memory-related brain regions can be relatively resistant to chemotherapy. One account of these observations is that the enduring deficits characterizing chemofog predominantly target cognitive capacities other than memory, such as information processing speed and executive function. The lack of an enduring impairment after chemotherapy in the present experiments may not generalize across all treatments and assays however. Deficits in novel object recognition have been reported 3 months after chemotherapy treatment (Fardell et al., 2010; Mondie et al., 2010) and in water maze probe trial performance 4 months after treatment (Fardell et al., 2010). Further study in animal models is also needed to test the possibility that cancer itself is a necessary precondition for the development of chemotherapy associated cognitive impairment. The degree to which the clinical syndrome known as chemofog reflects normal age-related cognitive decline that is attributed to cancer and its treatment also merits investigation.

The clinical profile of chemofog is increasingly understood to include deficits that persist or emerge beyond the completion of therapy, well after the acute side effects of treatment have resolved. In a recent study of breast cancer survivors, for example, adjuvant

Fig. 5. 14-unit T-maze performance. Rats showed rapid acquisition as reflected in a decrease in errors across trials. CYP/5FU cocktail did not impair performance.

chemotherapy that included the same agents used here was associated with cognitive impairment in 61% of patients assessed more than 7 months after treatment (Wefel et al., 2010). Among these patients, 30% displayed no deficits during earlier assessments conducted more proximal to chemotherapy. By comparison, a majority of related work in animal models has evaluated the status of cognitive function soon after treatment, typically following just a week or two of recovery (Gandal et al., 2008; Liedke et al., 2009; Macleod et al., 2007; Madhyastha et al., 2002; Seigers and Fardell, 2011; Seigers et al., 2009; Winocur et al., 2006).

In an effort to more closely model the clinical condition, the current experiments targeted the persistence of potential impairment, evaluating learning and memory 8 weeks following the last round of drug administration, when signatures of acute toxicity had substantially subsided. Protracted recovery is a significant element of the experimental design because CYP/5FU is known to suppress bone marrow and reduce hematocrit for more than 2 weeks after administration (Branda et al., 2002), and low hematocrit is associated with deficits on both the water and 14-unit T-maze (Hengemihle et al., 1996; Spangler et al., 1995). Despite the 8-week recovery, body weight in rats given CYP/5FU remained lower than controls, raising the possibility that recovery from treatment was incomplete, and that task performance might suffer as a consequence.

In the current experiments, memory for fear conditioning acquired before treatment and assessed 5 months later was intact following dosing regimens of CYP/5FU cocktail that induced marked acute toxicity and enduring weight loss. These results confirm and extend a recent study where 1 and 14-day retention of contextual fear memory was normal in mice receiving a cocktail of methotrexate (MTX) and 5FU 3 weeks before training (Gandal et al., 2008). Outcomes across reports, however, have varied. In another investigation, rats that received a CYP plus doxorubicin cocktail 1 week before training displayed impaired 24-hour retention of conditioned fear for context, together with intact memory for cue conditioning (Macleod et al., 2007). Impairments were also observed in a recent report that assessed contextual fear memory 30 days after training, in rats given MTX just 1 h after conditioning (Seigers et al., 2009). Although the precise factors responsible for the differing results across studies remain to be specified, our findings add to growing evidence that remote memory impairment is not a necessary consequence of treatment with chemotherapeutic agents.

Results from parallel research using maze procedures to examine the cognitive effects of anticancer drug administration have been relatively consistent. In agreement with the data reported here, findings from multiple studies are compatible with the conclusion that acquisition and 24-hour retention of spatial learning in the Morris water maze is preserved after chemotherapy (Lee et al., 2006; Li et al., 2008; Seigers et al., 2008; Winocur et al., 2006). Preserved acquisition was also confirmed in a study using a more challenging 14-unit T-maze, and indeed in that case we observed an anomalous, transient benefit of CYP and 5FU on spatial learning (Lee et al., 2006). Against this background of sparing, at least some chemotherapy agents can impair longer-term maze retention, tested 7 (Seigers et al., 2009), 30 (Li et al., 2008) or 120 days (Fardell et al., 2010) after acquisition. In combination, these data imply that the influence of chemotherapy on learning and memory may be relatively selective, affecting certain processing capacities without substantially compromising overall function. This characterization is compatible with an emerging clinical literature suggesting that chemofog comprises a constellation of fairly subtle cognitive deficits with modest consequences on activities of daily living (Burstein, 2007).

There is reason to suspect that a more detailed, comprehensive animal model of cognitive impairment associated with chemotherapy will require an additional focus on information processing speed and other capacities. Indeed a growing clinical literature suggests that, irrespective of the presence or absence of deficits in memory per se, processing speed, attention and concentration can be prominently affected (Vardy et al., 2008). Emerging data from animals studies are consistent with this perspective (Foley et al., 2008; Konat et al., 2008a; Liedke et al., 2009; Madhyastha et al., 2002; Mustafa et al., 2008; Seigers and Fardell, 2011), and underscore the value of broad neuropsychological screening in advancing such models. Increased focus is also needed to define the factors that mediate risk for the development of chemofog, and that account for the substantial variability in cognitive outcomes observed among individuals receiving similar treatments. Cancer itself may be among these factors, and greater attention should be directed at testing whether the duration, severity and aggressiveness of disease modulate and predict the cognitive response to therapy. Future studies in animal models, where these variables can be systematically manipulated, will be critical in this effort.

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