Pharmacology, Biochemistry and Behavior 100 (2012) 451–457

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Recent and remote spatial memory in mice treated with cytosine arabinoside

Thane Fremouw [a,b,*], Christy L. Fessler [a], Robert J. Ferguson [c], Yamil Burguete [a]

[a] Dept. of Psychology, University of Maine, Orono, ME, United States
[b] Graduate School of Biomedical Sciences, University of Maine, Orono, ME, United States
[c] Clinical Research Center and Department of Rehabilitation Medicine, Eastern Maine Medical Center, Bangor, ME, United States

ARTICLE INFO

Article history:
Received 27 April 2011
Received in revised form 16 September 2011
Accepted 6 October 2011
Available online 12 October 2011

Keywords:
Cytosine arabinoside
Learning
Memory
Chemotherapy
Morris water maze
Chemo-brain
Chemo-fog

ABSTRACT

Clinical studies suggest that chemotherapy is associated with long-term cognitive impairment in some patients. A number of underlying mechanisms have been proposed, however, the etiology of chemotherapy-related cognitive dysfunction remains relatively unknown. As part of a multifaceted approach, animal models of chemotherapy-induced cognitive impairment are being developed. Thus far, the majority of animal studies have utilized a rat model, however, mice may prove particularly beneficial in studying genetic risk factors for developing chemotherapy-induced cognitive impairment. Various chemotherapy agents, including cytosine arabinoside (Ara-C), have been found to impair remote spatial memory in rats in the Morris water maze. The present study evaluated the effects of Ara-C on remote (30 d) spatial memory in mice. In addition, the possibility that time relative to chemotherapy treatment may modulate the effect of chemotherapy on spatial learning and/or recent (1 d) memory was explored. Male C57BL/6J mice received either Ara-C (275 mg/kg i.p. daily for 5 days) or saline. Spatial learning and memory was assessed using the Morris water maze. Half the mice performed a remote (30 d) memory version of the task and the other half performed a recent (1 d) memory version of the task. The experiment was designed such that the probe trial for the recent memory version occurred on the same day relative to chemotherapy treatment as the remote memory version. Despite significant toxic effects as assessed by weight loss, Ara-C treated mice performed as well as control mice during acquisition, recent memory, and remote memory portions of the task. As are some humans, C57BL/6J mice may be resistant to at least some aspects of chemotherapy induced cognitive decline.

1. Introduction

Adjuvant cancer chemotherapy has been linked to cognitive decline in cancer survivors. The cognitive impairment seems to occur across a diverse range of processes including working memory, attention, processing speed, concentration, and executive functions in cancer survivors (e.g., van Dam et al., 1998 and Vardy and Tannock, 2007, although see Raffa, 2010). These cognitive deficits are often temporary, however, for a subset of survivors, the deficits can last for years and can have a deleterious impact on survivor quality of life (Ahles et al., 2005; Ferguson and Ahles, 2003; Stanton, 2006; Tannock et al., 2004). Unfortunately, the etiology of chemotherapy-related cognitive dysfunction remains relatively unknown. Interactions between genetics, epigenetics, and the environment/life history of individuals, along with methodological and ethical issues, complicate studies of the cognitive effects of chemotherapy in humans. As a result, animal models of chemotherapy-induced cognitive dysfunction are being developed to compliment continued human research (Seigers and Fardell, 2011; Walker, 2010).

The majority of animal studies have examined the effects of 5-Fluorouracil, Cyclophosphamide, Doxorubicin, and/or Methotrexate and have shown that cognitive deficits occur in a variety of tasks after single agent or combination treatments (ElBeltagy et al., 2010; Fardell et al., 2010; Foley et al., 2008; Konat et al., 2008; MacLeod et al., 2007; Mustafa et al., 2008; Seigers et al., 2008; Seigers et al., 2009; Winocur et al., 2006; Yang et al., 2010; Yanovski et al., 1989). A common suggestion in those studies is that chemotherapy impairs hippocampal processing, likely because of a decline in neurogenesis (ElBeltagy et al., 2010; Mustafa et al., 2008; Seigers et al., 2008; Yang et al., 2010). Although the link between cognitive impairment, chemotherapy, and hippocampal neurogenesis appears repeatedly in the animal studies, there is growing evidence that damage to other brain regions (Fardell et al., 2010; Li et al., 2008; Winocur et al., 2006) or other cell types (Han et al., 2008; Seigers et al., 2009) can lead to impaired cognition as well.

In one such study, Li et al. (2008) found that cytosine arabinoside (Ara-C; an anti-metabolite used to treat hematological malignancies) caused a decrease in dendritic length, spine density, and branch points in the apical dendrites of pyramidal cells in the anterior cingulate cortex (ACC) of rats. In addition, Ara-C impaired remote (30 d), but not recent (1 d) spatial memory in the Morris water maze (MWM). Although the brain areas involved in processing memories

* Corresponding author at: Psychology Department, University of Maine, Orono, ME 04469-5742, United States. Tel.:+1 207 581 6128; fax: +1 207 581 5841.
E-mail address: thane.fremouw@umit.maine.edu (T. Fremouw).

0091-3057/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2011.10.008

of different durations remains unclear, it has been suggested that the ACC may play an important role for remote memories (Ding et al., 2008; Frankland et al., 2004; Restivo et al., 2009; Takehara et al., 2003; Teixeira et al., 2006). Thus, Li et al. (2008) concluded that Ara-C impairs long-term, but not short-term spatial memory, and that it does so, at least in part, via dendritic retraction in the ACC.

Various chemotherapeutic agents, including Ara-C, appear to cause a suppression of neuronal proliferation in the dentate gyrus (DG) (Dietrich et al., 2006; Han et al., 2008; Janelsins et al., 2010; Mignone and Weber, 2006; Seigers et al., 2008). Although the link between neurogenesis and memory has been controversial (Jaholkowski et al., 2009; Leuner et al., 2006), growing evidence suggests that neurogenesis plays a critical role in at least some memory functions (for reviews see Aimone et al., 2010; Deng et al., 2010; Koehl and Abrous, 2011; Leuner et al., 2006), including long-term spatial memories (Deng et al., 2009; Goodman et al., 2010; Trouche et al., 2009). Thus, the long-term spatial memory impairment seen in the Ara-C treated rats in Li et al. (2008) may have been caused, at least in part, by decreased neurogenesis in the DG, a possibility that Li et al. (2008) acknowledge they cannot rule out.

In addition to long-term spatial memory, recent results suggest hippocampal neurogenesis is also critical for spatial memory acquisition (Dupret et al., 2008; Farioli-Vecchioli et al., 2008; Zhang et al., 2008) and for relatively short-term spatial memory (Goodman et al., 2010) such as the 1 day memory tested in Li et al. (2008). As new adult DG cells develop, they undergo a number of morphological and physiological changes that allow some of them to survive and eventually integrate into the existing hippocampal circuit (for reviews see Aasebo et al., 2011; Duan et al., 2008; Kelsch et al., 2010; Lledo et al., 2006; Zhao et al., 2008). Maturation is probably best thought of as a dynamic, continuous function, however, at least 2 critical maturation time-windows have been suggested: one at ~1.5–3 weeks and another at ~4–6 weeks (Aasebo et al., 2011; Zhao et al., 2008). Although not fully understood yet, the maturation state of a DG cell may play an important role in how neurogenesis affects memory (Aasebo et al., 2011; Deng et al., 2010; Kee et al., 2007). Interestingly, as pointed out by Goodman et al. (2010), previous studies reporting a link between neurogenesis and spatial acquisition involved a decline in the population of DG cells that would have been at least 4 weeks old during acquisition of the spatial task (Dupret et al., 2008; Zhang et al., 2008). Thus, time relative to the decline in neural proliferation may play an important role in what type, or even if there is memory impairment.

As shown in Fig. 1A, the rats in the short-term version of the spatial memory task in Li et al. (2008) performed the memory recall test (the probe trial) 29 days earlier relative to the Ara-C treatment than the rats in the long-term group. Thus, an alternative interpretation of the Li et al. (2008) result is that Ara-C causes general, but delayed memory impairment, not impairment of just long-term spatial memory. That is, time since the chemotherapy treatment, not the duration of the memory (1 d vs 30 d) might have been the critical variable causing the memory impairment seen by Li et al. (2008).

To test that possibility, we modified the task used by Li et al. (2008) so that the time between the memory recall (the probe trial) and the Ara-C treatment was the same for both the short-term and the long-term memory groups as shown in Fig. 1B. This design also allowed us to explore the possibility that spatial acquisition might be impaired during this same time window.

A number of genetic mechanisms of chemotherapy-induced cognitive dysfunction have been proposed (for a review see Ahles and Saykin, 2007), thus the development of mouse models, with their associated genetic tools, should aid in the testing of those hypotheses. However, few such models have been tested in mice and the results have been mixed (Foley et al., 2008; Gandal et al., 2008; Mondie et al., 2010; Walker et al., 2011; Winocur et al., 2006; Yang et al., 2010). Thus, an additional goal of our study was to replicate, in mice, the original long-term memory impairment following Ara-C treatment in rats (Li et al., 2008) to develop a mouse model of chemotherapy-induced cognitive dysfunction.

2. Materials and methods

2.1. Animals

Sixty male C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME) at 8 weeks of age. Mice were housed 3–4 animals per cage with food and water provided ad libitum. A 12:12 light dark cycle was used (light on: 7:30 am–7:30 pm). All experiments were conducted in accordance with the "Principles of laboratory animal care" (NIH publication No. 86–23, revised 1985) and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Maine.

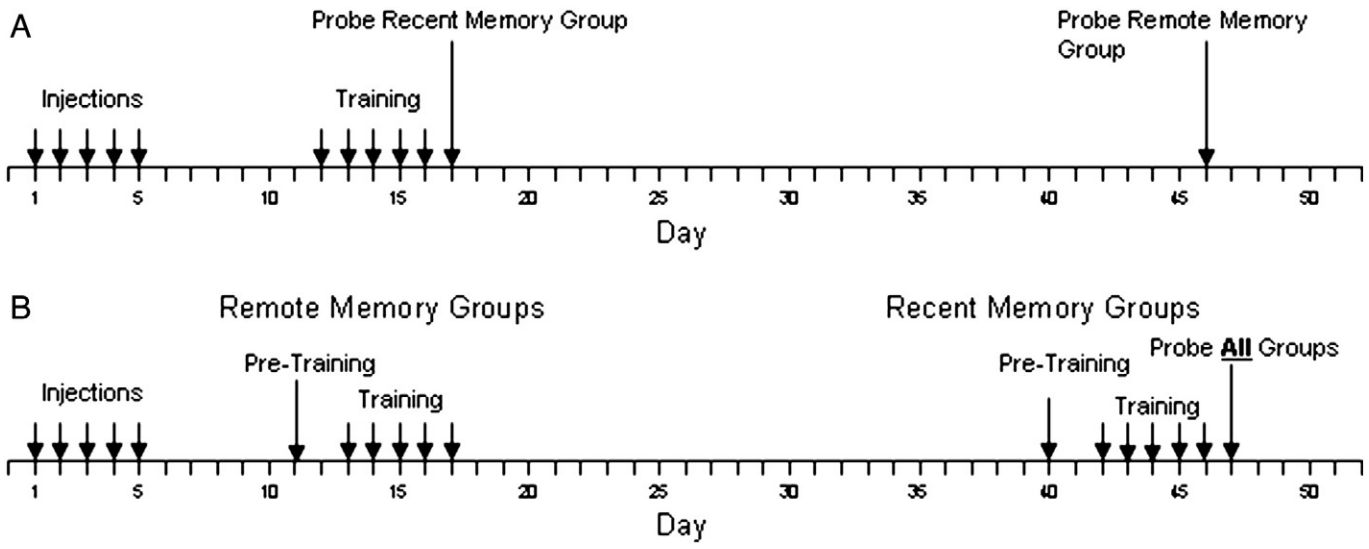

Fig. 1. (A) Time-line for Li et al. (2008). (B) Time-line for the present study.

2.2. Drug treatment

Following a 10 day acclimation period, mice received five daily intraperitoneal injections of saline (0.9% sterile saline, $n=30$) or cytosine arabinoside (Ara-C) (275 mg/kg dissolved in saline, $n=30$; Sigma-Aldrich, C1768). Because toxicity and tolerance of cancer chemotherapy agents can vary drastically as a function of circadian rhythm (Focan, 1995), we treated all mice at approximately the same time: 8 h after light onset. We note that Li et al. (2008) used 400 mg/kg per day for 5 days in their rats, however, preliminary dosing studies indicated that doses higher than 275 mg/kg resulted in significant animal death in our C57BL/6J mice. Starting 2 days before treatment and continuing 8 days after the last drug treatment, the mice were weighed daily. Thereafter, mice were weighed at least every third day. Two vehicle injected mice were excluded from the study after the injections had begun, but before behavioral training began (they were overly aggressive toward the other 3 mice in their cage and were therefore individually housed which would have been a potential confound).

2.3. Morris water maze (MWM)

Our goal was 1) to compare both remote and recent memory at the same time-point after Ara-C treatment, as measured by performance on a probe trial in a MWM procedure and 2) to measure spatial acquisition of the MWM at ~2 weeks and ~5 weeks after Ara-C treatment. To do so, mice in each treatment group (Ara-C or Saline) were randomly divided to perform either a recent memory (Ara-C treated $n=15$ and saline treated $n=14$) or a remote memory (Ara-C treated $n=15$ and saline treated $n=14$) version of the MWM task. In the recent memory version, mice performed the probe trial 1 day after completion of training. In the remote memory version, mice performed the probe trial 30 days after completion of training. Mice in the remote memory version began pre-training 6 days after the last treatment injection and mice in the recent memory version began pre-training 35 days after the last treatment such that the probe trial was 42 days after the last treatment injection for both groups. Fig. 1B illustrates the timeline for the two versions of the MWM procedure used here. Other than the start point for training and the delay between completion of training and the probe trial, the procedure was the same for both versions of the task as described in the rest of this Section 2.3.

All mice were individually handled for 5 min a day for 3 days and then run on a 1 day pre-training protocol to acclimate them to various aspects of the task (e.g., swimming, finding a hidden platform, and climbing onto the hidden platform). Pre-training consisted of 5 trials in a semi-opaque plastic rectangular box (71 cm × 46 cm × 33 cm) located in a different room than used for the MWM task. The pre-training box was filled with water (22 ± 1.5 °C) made opaque with white non-toxic tempura paint. A hidden circular escape platform (11 cm in diameter) sat ~1.5 cm below the water surface at the far end of the pre-training box. On each trial, the mouse was placed in the pre-training box and allowed to search for the escape platform. A trial continued until the mouse climbed onto the platform or 120 s had elapsed. If the mouse did not find the platform within 120 s it was guided to the platform and stayed on the platform for 10 s. At the completion of the trial, the mouse was placed in a cage under a lamp until the next trial. A cloth towel was draped over the cage to block the light from the lamp. Mice were run in groups of 2–3 resulting in an inter-trial interval of between 1 and 6 min.

Two days after pre-training, mice were trained on the MWM task. The MWM task was conducted in a blue circular pool (159 cm in diameter and 58 cm deep). The pool was filled with water (22 ± 1.5 °C) to a depth of 51 cm. White non-toxic tempura paint was added to the water to make it opaque. A circular escape platform (15 cm in diameter) was submerged ~1.5 cm below the water surface in the center of the NE quadrant. Multiple cues (pictures and black geometric shapes) were attached to the walls. The room was lit from above with fluorescent lighting that was partially dimmed by spray-painting the light covers (130 lux). Path data was collected via an automated tracking system (Any-Maze, Stoelting).

Training consisted of 8 trials a day broken into 2 blocks of 4 trials (~3 h between blocks) for 5 consecutive days (a total of 40 training trials). On each trial, the mouse was placed in the pool, with its head facing the wall, at 1 of the 4 cardinal compass positions (N, S, E, or W). Starting locations were chosen pseudo-randomly with the constraint that each of the cardinal positions was used in each block. A trial continued until the mouse climbed onto the platform or 60 s had elapsed. If the mouse did not find the platform within 60 s it was guided to the platform. Animals remained on the platform for 10 s at the end of each trial. At the completion of the trial, the mouse was placed in a cage under a lamp until the next trial. A cloth towel was draped over the cage to block the light from the lamp. Mice were run in groups of 2 resulting in an inter-trial interval of between 1 and 4 min. After the completion of training (40 trials), a probe test was run to assess spatial memory. The escape platform was removed and the mouse was allowed to swim for 60 s.

3. Results

3.1. Effect of Ara-C on weight

As shown in Fig. 2, the Ara-C treated mice lost ~9% of their pre-treatment weight by the 7th or 8th day after treatment began, while the vehicle treated mice gained ~2% of their pre-treatment weight over that same time period. The Ara-C treated mice had gained back the majority of their weight-loss by day 12. A 3-way repeated-measures ANOVA [Treatment (Saline or Ara-C) × Task-Version (Recent or Remote) × Day (1–5)] confirmed that the Ara-C and saline treated mice were significantly different than each other (main effect of Treatment, $F_{1,\ 54}=55.9$; $p<0.001$). There was also a Treatment × Day interaction ($F_{10,\ 540}=29.8$; $p<0.001$) indicative of the fact that the Ara-C animals lost weight and then gained weight whereas the saline animals slowly gained weight over the 12 days. There was no Treatment by Task-Version interaction ($F_{1,\ 54}=0.04$; $p>0.84$) indicative of the fact that the two Ara-C groups (recent and remote) had similar weight patterns and that the two saline groups had similar weight patterns. Mean weight did not differ between the 4 groups on the first day

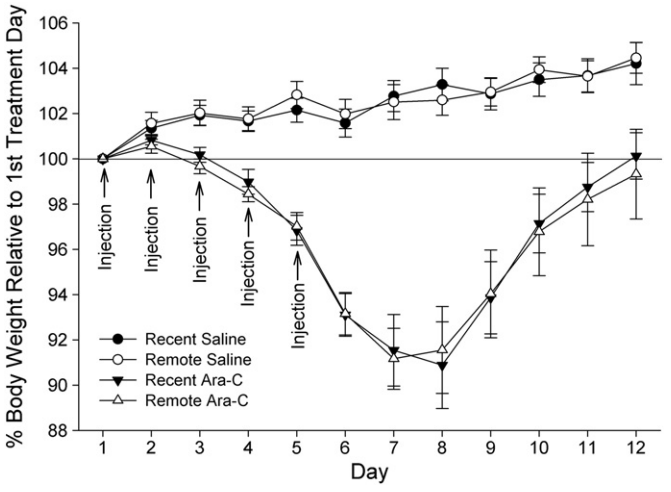

Fig. 2. Mean % body weight (relative to pre-treatment weight on the first day of treatment) as a function of memory group (recent or remote) and treatment (saline or Ara-C). Ara-C and saline treated animals were significantly different [$F_{1,\ 54}=55.9$; $p<0.001$]. Error bars represent ± standard error of the mean.

of treatment (25.9 ± 0.3, 25.1 ± 0.4, 25.3 ± 0.4, and 25.4 ± 0.4 g, for the Saline–Remote, Saline–Recent, Ara-C–Remote, and Ara-C–Recent groups, respectively: $F_{3,\ 57} = 0.71$; $p > 0.5$).

3.2. Effect of Ara-C on water maze training

As shown in Fig. 3, latency to find the hidden platform decreased over the training period and was similar for all 4 groups. A 3-way repeated-measures ANOVA [Treatment (Saline or Ara-C) × Task-Version (Recent or Remote) × Day (1–5)] confirmed that latency decreased over days (main effect of Day, $F_{4,\ 216} = 74.63$; $p < 0.001$) and confirmed that there were no significant differences between the Ara-C treated mice and the saline control mice in terms of latency to find the hidden platform during training (no main effect of Treatment, $F_{1,\ 54} = 0.02$; $p > 0.89$, and $p > 0.54$ for all interactions involving Treatment). The pattern of results was the same for distance traveled (data not shown) and on a finer time scale (1/2 day blocks; data not shown).

3.3. Effect of Ara-C on Morris water maze recall

Either 1 day (recent memory group) or 30 days (remote memory group) after the last training trial, spatial memory was assessed with a probe trial. Fig. 4A shows performance during the probe trial assessed with what has been reported to be the most sensitive (Maei et al., 2009) of the common probe measures: Gallagher's measure of proximity (Gallagher et al., 1993). There was no significant difference between the vehicle control group and the Ara-C treated group for either version of the task (recent: $t_{27} = 0.53$, $p = 0.60$, remote: $t_{27} = -0.10$, $p = 0.92$).

Because we found no difference between the vehicle and Ara-C treated group on the remote version of the task and hence failed to replicate the remote spatial memory impairment seen by Li et al. (2008), we examined performance during the probe trial using a number of other commonly used probe measures.

Fig. 4B shows performance during the probe trial assessed by percent time spent in the platform quadrant, the most common measure (Maei et al., 2009) of probe performance in the water maze task and the measure used in Li et al. (2008). There was no significant difference between the saline control group and the Ara-C treated group for either version of the task (recent: $t_{27} = 0.25$, $p = 0.80$, remote: $t_{27} = -0.78$, $p = 0.44$).

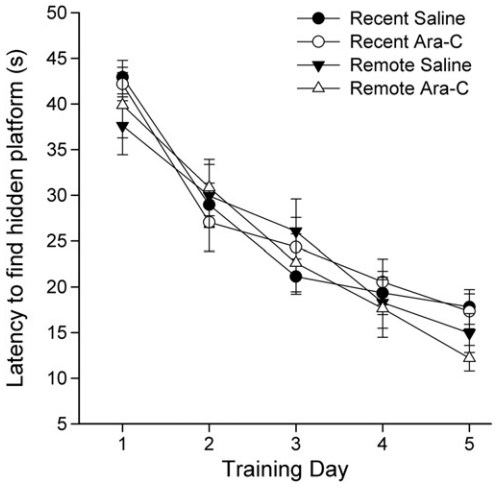

Fig. 3. Mean latency (s) to find the hidden platform as a function of memory group (recent or remote) and treatment (saline or Ara-C) blocked over days. Latency decreased over days [$F_{4,\ 216} = 74.63$; $p < 0.001$], but no differences were found involving treatment (all $ps > 0.54$). Error bars represent ± standard error of the mean.

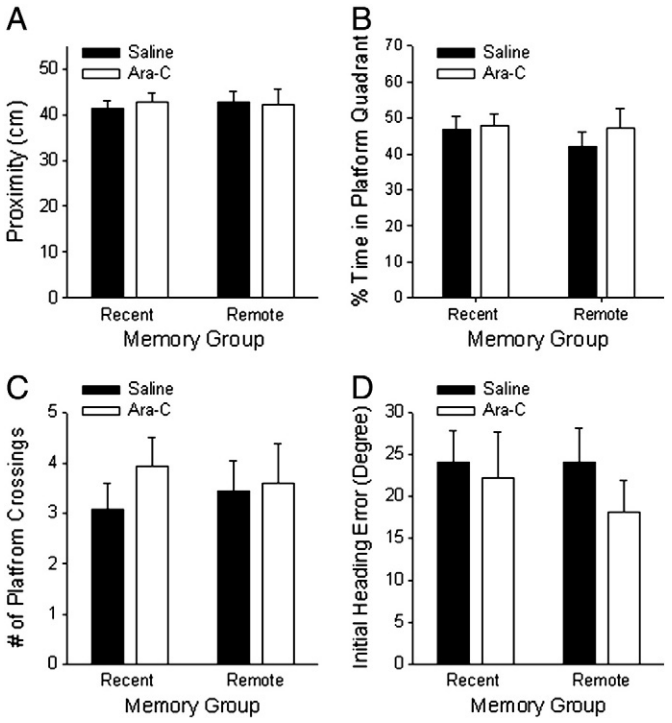

Fig. 4. Probe trial results as a function of memory group (recent or remote) and treatment (saline or Ara-C). (A) Gallager's proximity measure (Gallagher et al., 1993). (B) Mean % time spent in quadrant in which the hidden platform had been. (C) Mean # of crossings through the location where the hidden platform had been. (D) Mean initial heading error relative to the location where the platform had been. No significant differences were found (all $ps > 0.29$). Error bars represent ± standard error of the mean.

Fig. 4C shows performance during the probe trial assessed by number of crossings over the location where the platform had been during training, the second most common measure of performance in the water maze task during the probe trial (Maei et al., 2009). There was no significant difference between the saline control group and the Ara-C treated group for either version of the task (recent: $t_{27} = 1.08$, $p = 0.29$, remote: $t_{27} = 0.17$, $p = 0.86$).

Thus, using the 2 most common measures of performance during the probe trial (percent time platform quadrant and platform crossings) and what is thought to be the most sensitive probe measure (proximity), we found no evidence that Ara-C treatment caused impairment during the probe portion of the MWM task on either the remote or recent version of the task. Thus, we failed to replicate the remote spatial memory impairment following Ara-C treatment found by Li et al. (2008).

Because we failed to replicate Li et al. (2008) we looked at one additional measure of probe performance (initial heading; Fig. 4D) thought to assess slightly different cognitive processing than the measures used above (Vorhees et al., 2000; Vorhees and Williams, 2006). As with the previous measures, there was no significant difference between the saline control group and the Ara-C treated group for either version of the task (recent: $t_{27} = -0.35$, $p = 0.72$, remote: $t_{27} = -1.07$, $p = 0.29$).

4. Discussion

In the present study, our goal was 1) to replicate, using mice, the Ara-C induced long-term memory deficit found by Li et al. (2008) using rats and 2) to explore the possibility that short-term memory and/or acquisition in the MWM might be impaired by Ara-C when those components of the MWM occurred at the same time-point

relative to Ara-C treatment as used by Li et al. (2008) for the 30 d memory test. Mice injected with Ara-C lost significant weight compared to controls, yet performed as well as controls on the long-term (30 d) memory probe. Hence we were unable to replicate the long-term spatial memory impairment found by Li et al. (2008) (see also Fardell et al., 2010 for a remote spatial memory impairment in rats using the MWM task following methotrexate treatment). In addition, mice injected with Ara-C performed as well as controls in acquiring the MWM at 2 weeks (the remote memory group) and 5 weeks post-treatment (the recent memory group). Finally, Ara-C treated mice had unimpaired short-term (1 d) spatial memory when training occurred later (5–6 weeks after the last treatment) than in Li et al. (2008) (1–2 weeks after the last treatment). Thus, we found no evidence that Ara-C treatment in mice impairs memory in the MWM.

We believe it is important to note that with one exception, performance by Ara-C treated mice was numerically better on all probe trials (recent and remote) and for all probe measures (proximity, % time in platform quadrant, annulus crossings, and initial heading). In the one case for which the Ara-C treated mice were numerically worse than controls (recent memory probe assessed via proximity), the difference was small (1.5 cm) and a power analysis ($1\text{-}\beta = 0.85$, $\alpha = 0.05$, $d = 0.203$) indicated that 435 subjects per group would have been needed to detect a difference of that magnitude. In addition, during acquisition, the Ara-C treated animals often performed numerically better than the control animals. In fact, on day 5, both the remote and recent Ara-C treated groups performed numerically slightly better than their respective control group. Thus, there were no trends in any of our analyses towards the Ara-C animals performing worse than the control animals.

It is possible that methodological differences account for why we failed to replicate the long-term spatial memory deficit following Ara-C treatment seen in the Li et al. (2008) study. Perhaps the most obvious difference is species. Li et al. (2008) used rats whereas we used mice. Li et al. (2008) suggested that Ara-C caused a remote spatial memory deficit because it damaged the ACC. It is possible that the ACC plays a different role in remote spatial memory in mice than rats, although previous studies suggest ACC processing plays an important role in remote memories in both rats (Takehara et al., 2003) and mice (Ding et al., 2008; Frankland et al., 2004; Teixeira et al., 2006). However, to our knowledge, its role specifically in remote spatial memory has only been directly tested in mice (Teixeira et al., 2006). It is also possible that Ara-C does not cause damage to the ACC in mice or that it causes less damage in mice.

Our study and the Li et al. (2008) study also differed slightly in that we employed a day of pre-training in a separate room using a small square box to acclimate the mice to the task. It has been suggested that pre-training over two days with a visible platform (we used a hidden platform) using the same swim tank in the same room (we used a much different and smaller tank in a different room) can modify how neurogenesis plays a role in spatial memory (Zhang et al., 2008, although see Dupret et al., 2008). In addition, our training for the remote memory group occurred 1 day later relative to the last Ara-C treatment than in Li et al. (2008). Hence our remote probe trial occurred 1 day later as well. Although we believe it unlikely, we cannot rule out the possibility that those differences played a role in our failure to replicate the remote memory impairment seen in Li et al. (2008).

It is possible that a number of other methodological differences played a role in our inability to replicate the long-term memory impairment seen by Li et al. (2008). Our water temperature was 22 °C and theirs was 25 °C, the diameter of our tank was 159 cm and theirs was 210 cm, our intertrial interval was on average 3 min compared to 5 min in their study, and the wall cues and distance from the tank to the wall cues were probably different between the two studies.

Dosing also differed between the studies. Li et al. (2008) used a dose of 400 mg/kg per day for 5 consecutive days whereas we used a dose of 275 mg/kg per day for 5 consecutive days at approximately the same time: 8 ± 0.5 h after light onset (Li et al., 2008 did not report time of treatment). Theories on how to best calculate dose equivalencies between species vary to some extent (Reagan-Shaw et al., 2008; Rhomberg et al., 2007; Schneider et al., 2004), however, in general, a mouse mg/kg equivalent dose would be expected to be higher not lower (Freireich et al., 1966; Reagan-Shaw et al., 2008). However, pilot dosing studies indicated that doses higher than 275 mg/kg resulted in significant animal death in our mice (C57BL/6J). At 400 mg/kg, 66% (4/6) of the pilot animals died within 2 weeks of treatment. At 300 mg/kg, 25% (1/4) of the pilot animals died within 2 weeks of treatment. Thus, we used the highest dose possible that did not lead to systematic animal death. We note that Li et al. (2008) reported that all 20 of their Ara-C treated animals survived. In addition, our Ara-C treated mice showed significant weight loss (~9%) compared to weight gain (~2%) in the control mice suggesting that the treatment had a toxic effect (Stentoft, 1990). Li et al. (2008) did not report weight loss, however, Koros et al. (2007) reported no weight loss in rats after they administered Ara-C using the exact same dosage as Li et al. (2008): 400 mg/kg per day for 5 consecutive days. Given that higher doses caused systematic animal death and that the dose we used caused significant weight loss we believe it is unlikely that we failed to replicate Li et al. (2008) due to a low dose issue. Nevertheless, we cannot rule out the possibility that Ara-C would have resulted in cognitive impairment at a dose that causes systematic animal death, or for that matter, at a dose that does not cause weight loss. The lower tolerance to Ara-C treatment seen in our study may be a result of both species differences and time of treatment; toxicity and tolerance to cancer chemotherapy agents can vary drastically as a function of circadian rhythm (Focan, 1995). We believe it is important for time of treatment to be listed in all future studies in this area.

Given that multiple studies have shown that neurogenesis plays a critical role in remote spatial memory (Deng et al., 2009; Goodman et al., 2010; Trouche et al., 2009) and that multiple studies have shown that chemotherapy, including Ara-C, can cause a decline in neurogenesis (e.g., Dietrich et al., 2006; ElBeltagy et al., 2010; Han et al., 2008; Janelsins et al., 2010; Mignone and Weber, 2006; Seigers et al., 2008), it is also possible that a decline in neurogenesis, perhaps in combination with ACC damage, caused the deficit seen in Li et al. (2008). On a gross level, chemotherapy seems to cause similar damage in rats and mice: a decline in neurogenesis (Dietrich et al., 2006; ElBeltagy et al., 2010; Han et al., 2008; Janelsins et al., 2010; Mignone and Weber, 2006; Seigers et al., 2008) and white matter damage (Han et al., 2008; Seigers et al., 2008). It is possible, however, that there are more subtle differences, perhaps in the extent of neurogenesis decline or the amount of white matter damage, between species or between strains within a species. It is interesting to note that neurogenesis levels vary greatly between mouse strains (Clark et al., 2011; Kempermann et al., 2006; Kempermann and Gage, 2002a,b). Under standard housing conditions, the C57BL/6J strain, used in the present study, has a high level of neurogenesis (Clark et al., 2011; Kempermann et al., 2006; Kempermann et al., 1997) compared to numerous other strains. It is unclear why C57BL/6J mice have a high baseline level of neurogenesis compared to other strains, but one of many possibilities is that C57BL/6J mice are less susceptible to damage at one or more stages along the maturation path. Thus, it is possible that C57BL/6J mice are more resilient, as assessed via changes in neurogenesis, to chemotherapy than other strains. Interestingly, Gandal et al. (2008) found no impairment in C57BL/6 mice treated with methotrexate and 5-fluorouracil on two tasks for which rats have been reported to have an impairment: contextual fear conditioning and novel object recognition (although see Mondie et al., 2010 for a novel object recognition impairment

following thioTEPA in C57BL/6J mice). Strain differences in susceptibility to cognitive or neuroanatomical changes following chemotherapy might be an important avenue for future research.

Although we failed to replicate the remote spatial memory impairment seen by Li et al. (2008), our results are consistent with their results in terms of recent spatial memory and spatial acquisition. Time between Ara-C treatment and training varied between the two studies, yet in both studies there was neither indication that Ara-C impaired acquisition of the MWM task nor that it impaired recent spatial memory. Although we cannot rule out the possibility that there is some time-window during which Ara-C treatment impairs recent spatial memory or spatial acquisition, combined, the two studies argue against it, and are consistent with the broader notion that chemotherapy in general does not impair recent spatial memory or spatial acquisition (Fardell et al., 2010; Lee et al., 2006; Winocur et al., 2006). An alternative interpretation of our results is that methodological differences that often vary slightly among MWM tasks, for example, an intertrial interval of 3 min vs 5 min, or a probe trial on day 46 rather than 47, may play a critical role in whether Ara-C treatment causes impaired long-term spatial memory. If so, the MWM may not be a stable task for the development of a mouse model of chemotherapy induced cognitive dysfunction.

In summary, using a mouse model, we found no evidence that Ara-C treatment impaired acquisition, short-term memory, or long-term memory on a MWM task at a dose that caused systemic toxicity and was near the upper threshold of survivability. Although progress has been made (for reviews see Seigers and Fardell, 2011 and Walker, 2010), we believe the development of animal models of chemotherapy induced cognitive impairment will take time. Although there is strong evidence that chemotherapy causes a decline in neurogenesis (Dietrich et al., 2006; Han et al., 2008; Janelsins et al., 2010; Mignone and Weber, 2006; Seigers et al., 2008), how such a decline might affect cognition remains controversial (Jaholkowski et al., 2009; Leuner et al., 2006) and it is unlikely that a decline in neurogenesis alone can account for the wide range of processes (e.g., working memory, attention, concentration, processing speed, and executive functions) that appear to be affected in the human condition (e.g., van Dam et al., 1998; Vardy and Tannock, 2007). As has been previously suggested (e.g., Seigers and Fardell, 2011), inclusion of tasks thought to tap into executive function and attention may prove particularly fruitful as may the exploration of chemotherapy induced myelin damage.

Acknowledgments

We would like to thank Erin Sweeney for her excellent behavioral help. This research was funded in part by a grant from the Maine Institute for Human Genetics and Health. The Maine Institute for Human Genetics and Health had no involvement in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

References

Aasebo IE, Blankvoort S, Tashiro A. Critical maturational period of new neurons in adult dentate gyrus for their involvement in memory formation. Eur J Neurosci 2011;33:1094–100.
Ahles TA, Saykin AJ. Candidate mechanisms for chemotherapy-induced cognitive changes. Nat Rev Cancer 2007;7:192–201.
Ahles TA, Saykin AJ, Furstenberg CT, Cole B, Mott LA, Titus-Ernstoff L, et al. Quality of life of long-term survivors of breast cancer and lymphoma treated with standard-dose chemotherapy or local therapy. J Clin Oncol 2005;23:4399–405.
Aimone JB, Deng W, Gage FH. Adult neurogenesis: integrating theories and separating functions. Trends Cogn Sci 2010;14:325–37.
Clark PJ, Kohman RA, Miller DS, Bhattacharya TK, Brzezinska WJ, Rhodes JS. Genetic influences on exercise-induced adult hippocampal neurogenesis across 12 divergent mouse strains. Genes Brain Behav 2011;10:345–53.
Deng W, Aimone JB, Gage FH. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat Rev Neurosci 2010;11:339–50.
Deng W, Saxe MD, Gallina IS, Gage FH. Adult-born hippocampal dentate granule cells undergoing maturation modulate learning and memory in the brain. J Neurosci Off J Soc Neurosci 2009;29:13532–42.
Dietrich J, Han R, Yang Y, Mayer-Proschel M, Noble M. CNS progenitor cells and oligodendrocytes are targets of chemotherapeutic agents in vitro and in vivo. J Biol 2006;5:22.
Ding HK, Teixeira CM, Frankland PW. Inactivation of the anterior cingulate cortex blocks expression of remote, but not recent, conditioned taste aversion memory. Learn Mem 2008;15:290–3.
Duan X, Kang E, Liu CY, Ming GL, Song H. Development of neural stem cell in the adult brain. Curr Opin Neurobiol 2008;18:108–15.
Dupret D, Revest JM, Koehl M, Ichas F, De Giorgi F, Costet P, et al. Spatial relational memory requires hippocampal adult neurogenesis. PLoS One 2008;3:e1959.
ElBeltagy M, Mustafa S, Umka J, Lyons L, Salman A, Chur-yoe GT, et al. Fluoxetine improves the memory deficits caused by the chemotherapy agent 5-fluorouracil. Behav Brain Res 2010;208:112–7.
Fardell JE, Vardy J, Logge W, Johnston I. Single high dose treatment with methotrexate causes long-lasting cognitive dysfunction in laboratory rodents. Pharmacol Biochem Behav 2010;97:333–9.
Farioli-Vecchioli S, Saraulli D, Costanzi M, Pacioni S, Cina I, Aceti M, et al. The timing of differentiation of adult hippocampal neurons is crucial for spatial memory. PLoS Biol 2008;6:e246.
Ferguson RJ, Ahles TA. Low neuropsychologic performance among adult cancer survivors treated with chemotherapy. Curr Neurol Neurosci Rep 2003;3:215–22.
Focan C. Circadian rhythms and cancer chemotherapy. Pharmacol Ther 1995;67:1-52.
Foley JJ, Raffa RB, Walker EA. Effects of chemotherapeutic agents 5-fluorouracil and methotrexate alone and combined in a mouse model of learning and memory. Psychopharmacology 2008;199:527–38.
Frankland PW, Bontempi B, Talton LE, Kaczmarek L, Silva AJ. The involvement of the anterior cingulate cortex in remote contextual fear memory. Science 2004;304:881–3.
Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother 1966;50:219–44.
Gallagher M, Burwell R, Burchinal M. Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. Behav Neurosci 1993;107:618–26.
Gandal MJ, Ehrlichman RS, Rudnick ND, Siegel SJ. A novel electrophysiological model of chemotherapy-induced cognitive impairments in mice. Neuroscience 2008;157:95-104.
Goodman T, Trouche S, Massou I, Verret L, Zerwas M, Roullet P, et al. Young hippocampal neurons are critical for recent and remote spatial memory in adult mice. Neuroscience 2010;171:769–78.
Han R, Yang YM, Dietrich J, Luebke A, Mayer-Proschel M, Noble M. Systemic 5-fluorouracil treatment causes a syndrome of delayed myelin destruction in the central nervous system. J Biol 2008;7:12.
Jaholkowski P, Kiryk A, Jedynak P, Ben Abdallah NM, Knapska E, Kowalczyk A, et al. New hippocampal neurons are not obligatory for memory formation; cyclin D2 knockout mice with no adult brain neurogenesis show learning. Learning & Memory 2009;16:439–51.
Janelsins MC, Roscoe JA, Berg MJ, Thompson BD, Gallagher MJ, Morrow GR, et al. IGF-1 partially restores chemotherapy-induced reductions in neural cell proliferation in adult C57BL/6 mice. Cancer Invest 2010;28:544–53.
Kee N, Teixeira CM, Wang AH, Frankland PW. Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. Nat Neurosci 2007;10:355–62.
Kelsch W, Sim S, Lois C. Watching synaptogenesis in the adult brain. Annu Rev Neurosci 2010;33:131–49.
Kempermann G, Chesler EJ, Lu L, Williams RW, Gage FH. Natural variation and genetic covariance in adult hippocampal neurogenesis. Proc Natl Acad Sci U S A 2006;103:780–5.
Kempermann G, Gage FH. Genetic determinants of adult hippocampal neurogenesis correlate with acquisition, but not probe trial performance, in the water maze task. Eur J Neurosci 2002a;16:129–36.
Kempermann G, Gage FH. Genetic influence on phenotypic differentiation in adult hippocampal neurogenesis. Brain Res Dev Brain Res 2002b;134:1-12.
Kempermann G, Kuhn HG, Gage FH. Genetic influence on neurogenesis in the dentate gyrus of adult mice. Proc Natl Acad Sci U S A 1997;94:10409–14.
Koehl M, Abrous DN. A new chapter in the field of memory: adult hippocampal neurogenesis. Eur J Neurosci 2011;33:1101–14.
Konat GW, Kraszpulski M, James I, Zhang H-T, Abraham J. Cognitive dysfunction induced by chronic administration of common cancer chemotherapeutics in rats. Metab Brain Dis 2008;23:325–33.
Koros C, Papalexi E, et al. Effects of AraC treatment on motor coordination and cerebellar cytoarchitecture in the adult rat. A possible protective role of NAC. Neurotoxicology 2007;28(1):83–92.
Lee GD, Longo DL, Wang Y, Rifkind JM, Abdul-Raman L, Mamczarz JA, et al. Transient improvement in cognitive function and synaptic plasticity in rats following cancer chemotherapy. Clin Cancer Res 2006;12:198–205.
Leuner B, Gould E, Shors TJ. Is there a link between adult neurogenesis and learning? Hippocampus 2006;16:216–24.
Li C, Liu D, Huang L, Wang H, Zhang J, Luo X. Cytosine arabinoside treatment impairs the remote spatial memory function and induces dendritic retraction in the anterior cingulate cortex of rats. Brain Res Bull 2008;77:237–40.

Lledo PM, Alonso M, Grubb MS. Adult neurogenesis and functional plasticity in neuronal circuits. Nat Rev Neurosci 2006;7:179–93.
MacLeod JE, DeLeo JA, Hickey WF, Ahles TA, Saykin AJ, Bucci DJ. Cancer chemotherapy impairs contextual but not cue-specific fear memory. Behav Brain Res 2007;181: 168–72.
Maei HR, Zaslavsky K, Teixeira CM, Frankland PW. What is the most sensitive measure of water maze probe test performance? Front Integr Neurosci 2009;3:4.
Mignone RG, Weber ET. Potent inhibition of cell proliferation in the hippocampal dentate gyrus of mice by the chemotherapeutic drug thioTEPA. Brain Res 2006;1111:26–9.
Mondie CM, Vandergrift KA, Wilson CL, Gulinello ME, Weber ET. The chemotherapy agent, thioTEPA, yields long-term impairment of hippocampal cell proliferation and memory deficits but not depression-related behaviors in mice. Behav Brain Res 2010;209:66–72.
Mustafa S, Walker A, Bennett G, Wigmore PM. 5-Fluorouracil chemotherapy affects spatial working memory and newborn neurons in the adult rat hippocampus. Eur J Neurosci 2008;28:323–30.
Raffa RB. Short introduction and history. Adv Exp Med Biol 2010;678:1-10.
Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J 2008;22:659–61.
Restivo L, Vetere G, Bontempi B, Ammassari-Teule M. The formation of recent and remote memory is associated with time-dependent formation of dendritic spines in the hippocampus and anterior cingulate cortex. J Neurosci Off J Soc Neurosci 2009;29:8206–14.
Rhomberg LR, Baetcke K, Blancato J, Bus J, Cohen S, Conolly R, et al. Issues in the design and interpretation of chronic toxicity and carcinogenicity studies in rodents: approaches to dose selection. Crit Rev Toxicol 2007;37:729–837.
Schneider K, Oltmanns J, Hassauer M. Allometric principles for interspecies extrapolation in toxicological risk assessment—empirical investigations. Regul Toxicol Pharmacol 2004;39:334–47.
Seigers R, Fardell JE. Neurobiological basis of chemotherapy-induced cognitive impairment: a review of rodent research. Neurosci Biobehav Rev 2011;35:729–41.
Seigers R, Schagen SB, Beerling W, Boogerd W, van Tellingen O, van Dam FS, et al. Long-lasting suppression of hippocampal cell proliferation and impaired cognitive performance by methotrexate in the rat. Behav Brain Res 2008;186:168–75.
Seigers R, Schagen SB, Coppens CM, van der Most PJ, van Dam FS, Koolhaas JM, et al. Methotrexate decreases hippocampal cell proliferation and induces memory deficits in rats. Behav Brain Res 2009;201:279–84.
Stanton AL. Psychosocial concerns and interventions for cancer survivors. J Clin Oncol 2006;24:5132–7.
Stentoft J. The toxicity of cytarabine. Drug Saf 1990;5:7-27.
Takehara K, Kawahara S, Kirino Y. Time-dependent reorganization of the brain components underlying memory retention in trace eyeblink conditioning. J Neurosci Off J Soc Neurosci 2003;23:9897–905.
Tannock IF, Ahles TA, Ganz PA, Van Dam FS. Cognitive impairment associated with chemotherapy for cancer: report of a workshop. J Clin Oncol 2004;22:2233–9.
Teixeira CM, Pomedli SR, Maei HR, Kee N, Frankland PW. Involvement of the anterior cingulate cortex in the expression of remote spatial memory. J Neurosci Off J Soc Neurosci 2006;26:7555–64.
Trouche S, Bontempi B, Roullet P, Rampon C. Recruitment of adult-generated neurons into functional hippocampal networks contributes to updating and strengthening of spatial memory. Proc Natl Acad Sci U S A 2009;106:5919–24.
van Dam FS, Schagen SB, Muller MJ, Boogerd W, vd Wall E, Droogleever Fortuyn ME, et al. Impairment of cognitive function in women receiving adjuvant treatment for high-risk breast cancer: high-dose versus standard-dose chemotherapy. J Natl Cancer Inst 1998;90:210–8.
Vardy J, Tannock I. Cognitive function after chemotherapy in adults with solid tumours. Crit Rev Oncol Hematol 2007;63:183–202.
Vorhees CV, Inman-Wood SL, Morford LL, Broening HW, Fukumura M, Moran MS. Adult learning deficits after neonatal exposure to D-methamphetamine: selective effects on spatial navigation and memory. J Neurosci Off J Soc Neurosci 2000;20:4732–9.
Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 2006;1:848–58.
Walker EA. Animal models. Adv Exp Med Biol 2010;678:138–46.
Walker EA, Foley JJ, Clark-Vetri R, Raffa RB. Effects of repeated administration of chemotherapeutic agents tamoxifen, methotrexate, and 5-fluorouracil on the acquisition and retention of a learned response in mice. Psychopharmacology. 2011.
Winocur G, Vardy J, Binns M, Kerr L, Tannock I. The effects of the anti-cancer drugs, methotrexate and 5-fluorouracil, on cognitive function in mice. Pharmacol Biochem Behav 2006;85:66–75.
Yang M, Kim JS, Song MS, Kim SH, Kang SS, Bae CS, et al. Cyclophosphamide impairs hippocampus-dependent learning and memory in adult mice: possible involvement of hippocampal neurogenesis in chemotherapy-induced memory deficits. Neurobiol Learn Mem 2010;93:487–94.
Yanovski JA, Packer RJ, Levine JD, Davidson TL, Micalizzi M, D'Angio G. An animal model to detect the neuropsychological toxicity of anticancer agents. Med Pediatr Oncol 1989;17:216–21.
Zhang CL, Zou Y, He W, Gage FH, Evans RM. A role for adult TLX-positive neural stem cells in learning and behaviour. Nature 2008;451:1004–7.
Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. Cell 2008;132:645–60.