Published in final edited form as:

Behav Brain Res. 2019 April 19; 362: 319–322. doi:10.1016/j.bbr.2019.01.007.

5-Fluorouracil impairs attention and dopamine release in rats

David P. Jarmolowicz^{a,b}, Rachel Gehringer^c, Shea M. Lemley^a, Michael J. Sofis^a, Sam Kaplan^a, Michael A. Johnson^{c,*}

^(a)Department of Applied Behavioral Science, University of Kansas, 1000 Sunnyside, Avenue, Lawrence, KS 66045

^(b)Cofrin Logan Center for Addiction Research and Treatment, University of Kansas, 1000 Sunnyside, Avenue, Lawrence, KS 66045

^(c)Department of Chemistry, 1140 ISB/CDS1, 1567 Irving Hill Road, room 1140, Lawrence, KS 66045

Abstract

Chemotherapy related cognitive impairment (CTRC; “chemobrain”) is a syndrome that is associated with the impairment of various aspects of cognition, including executive function, processing speed, and multitasking. The role of neurotransmitter release in the expression of cognitive impairments is not well known. In this work we employed a newly developed behavioral paradigm to measure attentional shifting, a fundamental component of executive function, in rats treated with 5-fluorouracil (5-FU), a commonly used cancer chemotherapy agent. We found that one and two weeks of 5-FU treatment significantly impaired attentional shifting compared to baseline, while saline treatment had no effect. Post-mortem analysis of these rats revealed that 5-FU caused a significant overall decrease in dopamine release as well. Collectively, these results demonstrate the feasibility of our attentional shifting paradigm for evaluating the cognitive effects of chemotherapy treatment. Moreover, these results support the need for additional studies to determine if impaired dopamine release plays a role in chemobrain.

Keywords

dopamine; chemobrain; executive function; 5-fluorouracil; voltammetry

Chemotherapy related cognitive impairment (CTRC; “chemobrain”) is a syndrome that is associated with the impairment of various aspects of cognition, including executive function, processing speed, and multitasking[1]. Recent reports have linked adjuvant chemotherapy treatment for cancer to chemobrain; for example, one study found that roughly one third of women treated for breast cancer with chemotherapy experience cognitive dysfunction[2]. The heterogeneity of symptoms associated with chemobrain suggests that chemotherapy may impair basic behavioral and neurobiological processes that undergird day-to-day functioning.

*Corresponding Author: Prof. Michael A. Johnson, 2030 Becker Drive, Room 100, Multidisciplinary Research Building, Lawrence, KS 66047-1620 johnsonm@ku.edu.

Declaration of Interest: none

The chemotherapy agent, 5-fluorouracil (5-FU), which has been used to treat cancers of the breast[3], cervix[4], colon[5], esophagus[6], pancreas[7], and stomach[8], as well as topically for certain skin conditions[9], has been associated with cognitive impairment both in patients[10] and animal models[11, 12]. The primary mechanism of action of 5-FU is inhibition of thymidylate synthase; thus, it blocks the synthesis of thymidine, which is necessary for DNA replication [13]. The ability of 5-FU to cross the blood brain barrier (BBB) may facilitate its central nervous system toxicity[14–16].

A potential consequence of treatment with chemotherapy agents like 5-FU is impairment of executive function. In a widely adopted model, Snyder and coworkers have defined executive function as “a set of cognitive control processes, mainly supported by the prefrontal cortex (PFC), which regulate lower level processes (e.g., perception, motor responses) and thereby enable self-regulation and self-directed behavior toward a goal, allowing us to break out of habits, make decisions and evaluate risks, plan for the future, prioritize and sequence our actions, and cope with novel situations”[17]. This model breaks executive function down into three correlated yet separable behavioral processes: shifting one’s attention between tasks (i.e., attention), updating working memory (i.e., working memory), and inhibiting pre-potent responses (i.e., inhibition) with behavioral processes both interactively and separately contributing to “executive function” (EF)[17].

Although the prefrontal cortex is considered to be a primary mediator of EF[18], recent evidence suggests that dysfunction of the dopamine system, including that associated with the striatum, may also contribute to CTSC [19, 20]. The striatum receives inputs from other brain regions, including the cortex, hippocampus, amygdala, and thalamus[21], and amplifies desired responses while concurrently suppressing unwanted responses[22]. Dopaminergic neurons project to the striatum from the ventral tegmental area and substantia nigra pars compacta[23]. Given the importance of these pathways in motivation and learning [22, 24], it is likely that dopamine signaling in general, and dopamine release alterations in particular, influence cognitive function[25].

In previous work, we found that rats treated with 5-FU lack inhibitory control[12]. Additionally, treatment with carboplatin, another commonly used chemotherapeutic agent associated with chemobrain[26], not only impaired working memory[12], but also decreased neurotransmitter release[19]. The current study tested effects of 5-FU on attention, a fundamental component of EF. We compared our behavioral results with electrically stimulated dopamine (DA) release, measured with fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes.

For the measurement of attention, sixteen male Wistar rats from Charles River (Raleigh, NC) were maintained on a 22-hr food restriction schedule. Rats earned food pellets (45mg, Bio-Serv, Frenchtown, NJ) during 1-hr experimental sessions and then received ad libitum food for the remainder of the 2-hr access period beginning approximately 10 minutes after session. Although rats were housed and fed in pairs, we planned to separate rats for feeding and/or housing if dominance relations developed (this did not occur). The rats were 5 months old at the beginning of the experiment. Water was freely available in the home cages, and cages were located in a colony room with a 12h:12h light–dark cycle. Sessions occurred

during the light phase of this cycle. All procedures were in accordance with the guidelines established by the University of Kansas Institutional Animal Care and Use Committee and National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Behavioral sessions were conducted in standard MedPC operant chambers (30.5cm long, 24.1cm wide, 29.2cm high; Med Associates, Inc., St. Albans, VT) illuminated by 28-V houselights centered on the back wall (26.7cm from the floor). Centered on the front wall, 1cm above the floor grid was a pellet receptacle (3cm×4cm) into which a pellet dispenser could dispense grain-based pellets. On the curved, rear wall were five side-by-side, nose-poke access openings (2.54 cm × 2.54 cm), each illuminated by a cue light and featuring infrared response recording. Nose-poke openings were 2.54 cm apart and 2 cm from the floor. These behavioral testing chambers were housed in sound attenuating cabinets with white noise fans.

Figure 1 outlines the workflow for each rat. Behavioral testing was performed a minimum of six out of every seven days, at approximately the same time each day, for four weeks. Over the course of at least two weeks, rats experienced 12–14 sessions on the final baseline-testing paradigm prior to the experimental phase. For the experimental phase, which lasted two weeks, rats were randomized into one of two groups: 5-FU [n=10] or biological saline (SAL) [n=6]. The experimental phase for each rat began with its first administration of 5-FU or SAL. A second administration occurred one week later and rats were tested for an additional week, at which point they were euthanized for neurochemical measurements.

Rats were tested on our modified 5-choice serial reaction time task. On this task, rats initiated trials by poking their noses in the pre-lit food receptacle. Next, a variable wait period (1 – 3 s; mean 2-s) passed prior to the target nose poke receptacle being illuminated for 1 s. A 5-s response period began with this light onset, with a correct response consisting of the rat poking his nose in the target receptacle before the response period elapsed. Early responses or responses to other targets were recorded as incorrect and terminated the trial. A failure to respond during the 5-s response period was recorded as incorrect. When a trial was terminated, the feeder light was lit in anticipation of the rat initiating the next trial. The target nose poke location was selected at random on the first trial, and remained the same for an average of 5 trials (3–7) prior to a new target location being randomly selected. Sessions lasted one hour, with the baseline phase continuing until rats met a pre-determined baseline stability criterion. Stability occurred if the mean reinforcers earned over the first 3 sessions (of the final 6 sessions) and the last 3 sessions did not deviate from the mean reinforcers for all 6 sessions by more than 10% and there was no visual evidence of a monotonic trend.

Procedures for the measurement of dopamine release with FSCV are similar to those employed previously[19, 27]. Briefly, rats were anesthetized by isoflurane inhalation, decapitated, and the brain was removed and placed in an ice bath of oxygenated artificial cerebrospinal fluid (aCSF), which consisted of 2.5 mM KCl, 126 mM NaCl, 1.2 mM NaH_2PO_4 , 25 mM NaHCO_3 , 2.4 mM CaCl_2 , 1.2 mM MgCl_2 , 20 mM HEPES, 11 mM D-glucose, and was adjusted to a pH of 7.4. The brain was glued to an aluminum block and coronal slices, 350 μm thick, were made with a Leica VT1000S vibratome (Leica

Biosystems, Buffalo Grove, IL). For a given recording session, a brain slice was placed in an RC-22 perfusion chamber mounted in a PH-1 heated platform with an SH-27B in-line solution heater (Warner Instruments, Hamden, CT). The perfusion aCSF was maintained at 34°C with a TC-344C dual channel temperature controller (Warner Instruments, Hamden, CT).

Carbon-fiber microelectrodes, constructed in-house as previously described [28], were positioned in striata with a micromanipulator. Dopamine release was measured with background-subtracted FSCV in which Tar Heel software, provided by the University of North Carolina, Chapel Hill Electronics Shop, was used. Cyclic voltammograms (CVs) were collected by holding the carbon-fiber working electrode at a potential of -0.4 V, linearly scanning up to $+1.4$ V, and back down to -0.4 V at a scan rate of 400 V/s. Ten CVs per second were obtained. Dopamine release was evoked by application of a 4 ms duration, 350 μ A current, biphasic pulse to stimulus electrodes positioned on either side of the working electrode. Stimulated release plots were created by converting the oxidation potentials to concentration by calibration against known standard dopamine solutions in a flow cell and plotting versus time. Measurements were obtained from one striatal brain slice per rat. In each slice, dopamine release was measured at four randomly selected locations within the dorsolateral, ventrolateral, dorsomedial, and ventromedial quadrants in the striatum [19]. Measurements were averaged to determine an overall value for each rat.

All injections took place within two hours following behavioral sessions. Rats received injections (5-FU or saline) twice over the course of treatment, with the second administration occurring exactly one week removed from the start of treatment. Both 5-FU and saline were administered by i.v. injection through the tail vein (25 mg/kg).

In examining the behavioral results, we found no statistically significant differences in the number of rewards earned at baseline ($t[14] = 1.496$, $p = 0.1568$); however, we observed slightly different levels between groups (5-FU mean = 179.68 , Saline mean = 145.56). As such, we converted our post treatment data to percent of baseline measures for comparison to help mitigate heterogeneities between individual rats. For each rat, we calculated the mean of the final six sessions of baseline to the mean of the six days between injections 1 and 2, and the six days of sessions after injection 2. The means of these two percent of baseline scored were compared using a 2 (group) $\times$ 2 (time point) repeated measures ANOVA. All statistical analyses were conducted using Graphpad Prism 7.0.

Figure 2 shows percent of baseline reinforcers earned per hour by rats in the saline (grey bars) and 5-FU (white bars) groups after one and two weeks of treatment. A 2×2 repeated measures ANOVA ($n = 6$ saline-treated and 10 5-FU-treated rats analyzed at 1 and 2 weeks of treatment) found a significant effect of group ($F[1,14] = 13.02$, $p = 0.003$) but not time point ($F[1,14] = 1.67$, $p = 0.217$) nor a time point by group interaction ($F[1,14] = 0.57$, $p = 0.464$). Follow-up comparisons using Sidak's multiple comparisons method found significant differences between groups during both the first ($p = 0.003$) and second ($p = 0.023$) weeks of treatment.

One week after the rats underwent behavioral analysis, they were sacrificed for analysis of dopamine release and uptake with FSCV. Figure 3A shows representative raw data of electrically stimulated dopamine release measured in the striatum. CVs (inset) confirm the presence of dopamine. Overall values for dopamine release in saline control and 5-FU treated rats were obtained by sampling random locations in different quadrants of the striatum (Figure 3B)[19]. The bars show the group means, with the error bars showing the standard deviations. We analyzed dopamine release by two-way ANOVA and found a significant main treatment effect ($p = 0.0004$, $F[2,48] = 9.16$, $n = 5$ saline-treated rats and 9 5-FU-treated rats). Note: After the behavioral experiments, we were unable to obtain data from one saline-treated and one 5-FU-treated rat. We observed neither a significant effect of striatal region on release, nor a significant interaction between treatment and region.

In this work, a single injection with 5-FU significantly impaired attentional shifting in rats, and this effect persisted a week after the second injection. *Post mortem* neurochemical analyses revealed that dopamine release, electrically evoked and measured with FSCV in the striatum, was diminished. Moreover, a significant correlation existed between $[DA]_{\max}$ and reinforcers earned by 5-FU treated rats, but not SAL treated rats. There are several points that we would like to make about these findings.

First, in conjunction with our prior findings [19, 29], the present findings suggest that chemotherapy agents impair executive function. Specifically, Miyake et al., (2000)[30] have delineated executive function as consisting of attentional updating, updating working memory, and inhibiting proponent responses. The present findings suggest that 5-FU impairs a crucial component of executive function (i.e., attention). Given our prior research demonstrating that carboplatin impairs memory[19] and that KU-32 prevents 5-FU related impairments in response inhibition[29], chemobrain may be characterized by impaired executive function. This conceptualization is consistent with findings from clinical populations, which characterize chemobrain using the same executive function model.

Second, the behavioral testing for the present study contained important design elements that may help guide future inquiry using preclinical models. For example, the present study obtained a stable baseline of responding prior to drug administration. This is important for two reasons: 1) Chemotherapy based cognitive impairment is a cognitive change phenomenon. As such, drug effect should be compared to premorbid functioning. 2) The development of the complex cognitive performances disrupted in clinical populations (e.g., executive function) requires considerable time in rodent models.

The striatum receives inputs from several brain regions, including the prefrontal cortex, and amplifies wanted responses and suppresses unwanted responses[21]. Although the role of dopamine release impairment in chemobrain is not known, it is possible that dysregulation of striatal signaling may contribute to impairments in cognitive and motor function by aberrant handling of these inputs. Consistent with this concept, we have previously shown that dopamine release is impaired along with working memory in rats treated with carboplatin[19]. However, it is also important to note that serotonin release was impaired as well, suggesting that carboplatin-mediated toxicity causes the impairment of multiple neurotransmitter systems. Additionally, previous work has shown that treatment with

doxorubicin, another commonly used chemotherapeutic agent, impairs glutamate clearance[31]. Clearly, more work is required to determine if and how impairments in the release of dopamine and other neurotransmitter systems affect executive function.

In conclusion, we measured attentional updating, a fundamental component of executive function, and electrically evoked dopamine release in Wistar rats that had received injections of 5-FU or saline vehicle. This work is important because it revealed significant decreases in attentional updating resulting from 5-FU treatment, demonstrating the feasibility of our behavioral approach. Additionally, to our knowledge, this is the first published work showing that 5-FU treatment decreases striatal dopamine release. Therefore, our studies: 1) support the concept that dopamine release impairments may influence cognitive impairment in chemobrain and 2) justify future experiments aimed at measuring transient dopamine release events in rats carrying out behavioral tasks aimed at evaluating executive function.

Acknowledgments

Funding: The authors acknowledge funding for this project from the University of Kansas to MAJ and DPJ, The KU Cancer Center (NIH P30 CA168524) to MAJ, The Hall Foundation to MAJ, and The KU Strategic Initiative Program (INS0075092KU) to DPJ.

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Highlights

- We measured attentional shifting and dopamine release in rats treated with 5-fluorouracil (5-FU)
- 5-FU injection resulted in impaired ability of rats to shift attention
- We also found a significant decrease in dopamine release in acutely harvested brain slices
- This work provides support for future *in vivo* studies aimed at measuring dopamine release in behaving 5-FU treated rats.

Figure 1. Workflow conducted on each rat.

We conducted a minimum two-week course of baseline testing followed immediately by two weeks of behavioral testing and concurrent 5-FU administration. We then carried out post-mortem electrochemical analyses with FSCV one week after the second injection.

Figure 2. Treatment with 5-FU impairs attentional shifting.

Rats were treated with 5-FU for two weeks. A 2×2 repeated measures ANOVA ($n = 6$ saline-treated and 10 5-FU-treated rats analyzed at 1 and 2 weeks of treatment) found a significant effect of group ($F[1,14] = 13.02$, $p = 0.003$) but not time point ($F[1,14] = 1.67$, $p = 0.217$) nor a time point by group interaction ($F[1,14] = 0.57$, $p = 0.464$). Follow-up comparisons using Sidak's multiple comparisons method found significant differences between groups during both the first ($p = 0.003$) and second ($p = 0.23$) weeks of treatment. Bars represent mean $\pm$ SEM.

Figure 3. Treatment with 5-FU decreases dopamine release.

Rats were treated with 5-FU for one week and sacrificed one week after the last injection.

(A) Representative raw data show the time course of dopamine release and uptake. The cyclic voltammograms (inset) confirm the presence of dopamine. (B) Pooled results obtained from the entire group of rats tested. The bars show the group means, with the error bars showing the standard deviations. We analyzed dopamine release by two-way analysis of variance (ANOVA), and found a significant main treatment effect ($p = 0.0004$, $F[2,48] = 9.16$, $n = 5$ saline-treated and 9 5-FU-treated rats). We observed neither a significant effect of striatal region on release, nor a significant interaction between treatment and region. Bars represent mean $\pm$ SEM. The approximate locations of the quadrants are provided in the image to the right (image taken from Kaplan *et al.*[19], with permission from the American Chemical Society).