CHEMOTHERAPY RELATED COGNITIVE IMPAIRMENT IN THE RAT

by

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ABSTRACT

CHEMOTHERAPY RELATED COGNITIVE IMPAIRMENT IN THE RAT

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Treatment for cancer has been indicated to negatively impact the quality of life for patients. Specifically, chemotherapy has been associated with fatigue, nausea, and peripheral neuropathy. More recently, chemotherapy has also been found to be related to cognitive impairment in various cognitive domains including working memory, information processing speed, and visual attention. At this time, the mechanisms underlying this impairment are not understood and there is currently no treatment for this condition. The purpose of this study was to model chemotherapy related cognitive impairments using an established test of attention in rats. While receiving the chemotherapeutic agent Taxol, animals were tested daily in the Five Choice Serial Reaction Time Task (5CSRTT), a task which requires animals to attend and respond to

a visually presented stimulus in order to obtain reinforcement. In addition, animals were tested for the development of peripheral neuropathy and alterations in IL-6 and IL-1β cytokine levels. The results indicate that Taxol treated animals developed mechanical sensitivity, but did not exhibit alterations in cytokine levels or cognitive impairments in the 5CSRTT. It is imperative to better understand chemotherapy related cognitive impairments, but at this time more work is needed to elucidate the causes of these decrements.

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CHAPTER 1

INTRODUCTION

Cancer is a life altering, deleterious condition that affects approximately 1 to 2 percent of the population (Pisani et al, 2002), and approximately 500,000 people die from cancer each year (Mantyh, 2002). Although many treatments currently exist that are effective for managing cancer, most of these approaches have injurious side effects. For instance, the side effects of chemotherapy include fatigue, nausea, peripheral neuropathy, and cognitive impairment (Wefel et al, 2004b; Meyers et al, 2005). Despite these effects, chemotherapy is the most commonly used treatment for cancer due to its efficacy in managing cancer.

While sensory side effects including fatigue and neuropathy have long been acknowledged, cognitive impairment has only recently been considered as a major side effect of chemotherapy. This impairment, often referred to as chemofog or chemobrain, is described as a subtle decline in general cognitive ability, leading to feelings of absentmindedness (Wefel et al, 2004b). Patients report forgetting to engage in daily activities (i.e. brushing teeth), consistently forgetting where items were placed, and a general sense of difficulty in maintaining concentration.

Several studies have found chemotherapy related cognitive impairments in cancer patients in the domains of working memory, executive function, processing speed, verbal fluency and verbal memory, and visuospatial memory (Ahles and Saykin,

2007; Berglund et al, 1991; Brezden et al, 2000; Cleeland, 2000; Falleti et al, 2005; Jansen et al, 2005; Olin, 2001; Phillips and Bernhard, 2003; Poppelreuter et al, 2004; Schagen et al, 1999; van Dam et al, 1998; Wieneke and Dienst, 1995). Further, the prevalence of this impairment ranges from 17 to 75 % of chemotherapy treated patients (Tannock et al, 2004) and is seen up to 10 years after chemotherapy treatment (Ahles et al, 2002).

The cause of this impairment is uncertain at this time, but many potential mechanisms have been proposed, including structural changes to the brain revealed using neuroimaging techniques (Saykin et al, 2003), DNA damage due to oxidative stress (Ahles and Saykin, 2007), anemia (Jansen et al, 2005; Massa et al, 2006), and hormonal changes (Jansen et al, 2005; Phillips and Bernhard, 2003). Another potential cause indicated in the literature is dysregulation of cytokines (Cleeland, 2000; Meyers and Abbruzzese, 1992; Meyers, Albitar, and Estey, 2005; Wilson et al, 2002). Cytokines are non-antibody proteins that are released by various cells, commonly macrophages and T-helper cells, in response to illness, threat, injury, age, or stress (Wilson et al, 2002). Cytokines are involved in a variety of immune system related activities, including activating and producing monocytes, secreting antibodies, chemotaxis, and phagocytosis (Miller et al, 2002). They are an imperative part of the immune system; however, dysregulation of specific cytokines can be detrimental to the body. In addition, the use of exogenous cytokines has been shown to have deleterious side effects.

For example, Interleukins (IL), the largest class of cytokines, have been shown to lead to neurodegeneration. Specifically, IL-1 has been found to lead to increased calcium influx in hippocampal neurons (Larson and Dunn, 2001; Wilson et al, 2002). This finding has been supported by research showing that animals exposed to exogenous IL-1β exhibit deficits in tasks requiring uncompromised spatial abilities, such as the Morris Water Maze (Danzer, 2001; Gibertini et al, 1995; Oitzl et al, 1993). IL-1 has also been associated with taste aversion, fever, fatigue, and disruption of operant performance and other conditioning paradigms (Aubert et al, 1995; Crestani et al, 1991; Jain et al, 2001). Further, IL-1 is thought to attack endothelial cells, leading to blood brain barrier (BBB) leakage (Lincinio et al, 1998). This allows cytokines to cross the BBB increasing the neurotoxicity of other cytokines. IL-2 treatment has been associated with decrements in the domains of executive function, information processing speed, and reaction time (Meyers et al, 2004; Meyers et al, 2005;). In an animal study, Heyser et al (1997) showed that IL-6 may lead to hippocampal interneuron damage, as well as degeneration of neurons in the frontal cortex, via excitotoxicity, leading to poor performance on a task of avoidance learning. This is supported in human studies that found IL-6 plasma levels correlate to decreased cognitive function, especially in areas of executive function (Wilson et al, 2002).

Interferons (INF) have also been associated with cognitive impairment. Valentine et al (1998) showed that chronic administration of INFα leads to alterations in dopamine and serotonin levels, and this chronic exposure may lead to memory loss and slowed reaction times. Loftis and Hauser (2004) found confusion and impaired

concentration, in addition to sickness behavior such as fatigue with INFα. Similar cognitive impairments have been found by other researchers using INF therapy (Licinio et al, 1998; Smith et al, 1988).

Tumor necrosis factor (TNF) is another cytokine that has been shown to contribute to cognitive impairment. Increased levels of TNFα have been found in patients with HIV-associated dementia (Glass et al, 1993; Rostasy et al, 2000) and in close temporal proximity to traumatic brain injury (Knoblach et al, 1999). Further, Suzuki et al (2006) recently conducted a study on elderly patients with diabetes mellitus and found that higher TNFα levels were observed in relation to declines on tests of executive function and delayed recall. Research indicates that TNF may exert its negative cognitive effects by causing damage to astrocytes and damaging the BBB and by leading to demyelination throughout the central nervous system (Akassoglou et al, 1998; Ellison and Merchant, 1991; Magnano et al, 2004).

Overall, the greatest density of cytokine receptors is found in the hippocampus and hypothalamus (Wilson et al, 2002). Dense receptors in the hypothalamus would explain the pyrogenic effects of cytokines (Larson and Dunn, 2001; Lincinio et al, 1998), and dense receptors in the hippocampus may explain the cognitive effects of altered spatial abilities in cytokine treated animals (Gibertini et al, 1995; Oitzl et al, 1993). In support of this idea, Casolini et al (2002) found that TNF leads to neurotoxic levels of glucocorticoids binding in the hippocampus.

Chemotherapeutic agents have been shown to lead to increases in cytokines. For example, treatment with the chemotherapeutic agent paclitaxel (Taxol) has been shown

to lead to increases in IL-1β (O'Brien et al, 1995) and IL-6 (Penson et al, 2000; Pusztai et al, 2004; Rabinowitz et al, 1993; Tsvaris et al, 2002). In further researching Taxol, Dina et al (2001) hypothesized that an increase in cytokines may be the result of activation of second messenger systems, especially protein kinase C and protein kinase A. Activation of these systems leads to expression of immunomodulatory genes, which then increases production of cytokines via macrophages and T cells (Zaks-Zilberman et al., 2001).

Currently, little research has addressed a means to treat cancer related cognitive impairment. Researchers have attempted to reproduce cognitive impairments in animal models with little success or conflicting results using chemotherapeutic agents. Borzan et al (2004) found peripheral neuropathy using the chemotherapeutic agent vincristine but failed to find alterations in sensorimotor gating. Macleod et al (2007) used cyclophosphamide and found a decrease in contextual, but not cue specific, fear. Shors et al (2002) used the anitmitotic agent methylazoxymethanol acetate to show decreases in fear conditioning. However, the authors failed to show changes in anxiety or the Morris Water maze. Reiriz et al (2006) treated mice with cyclophosphamide and found impairment in retention of an avoidance conditioning task. Surprisingly, Lee et al (2006) found improved performance in the Morris Water Maze seven weeks following treatment with cyclophosphamide.

The chemotherapeutic agent methotrexate (MTX) has been used repeatedly to study chemotherapy related cognitive impairments in animal models. For example, Stock et al (1995) used MTX and failed to find impairment in a test of a conditioned

taste aversion. This is in direct opposition to Yanovski (1989) who did find impairment in acquisition of a taste aversion task using neonatal MTX treatment. Sieklucka-Dziuba et al (1998) also found impairments in a conditioning task using MTX. Madhyastha et al (2002) found similar results in addition to finding structural hippocampal changes. Others have found impaired performance in the Morris Water Maze using MTX (Seigers et al, 2007; Winocur et al, 2006). Currently, the reason for these discrepancies is unclear. Development of an animal model of chemobrain would allow for further investigation of the mechanisms of this impairment as well as potential treatments.

One potential means to study cognitive impairment following chemotherapy treatment in an animal model is the Five Choice Serial Reaction Time Task (5CSRTT). The 5CSRTT is an established test of attentional mechanisms that is commonly used in rodents and primates. The test is sensitive to changes in sustained, divided, and selective attention, and has been used to study the interaction of attention and motivational drives, pharmaceutical effects, and brain lesions (Robbins, 2002). The paradigm is an operant procedure that requires the animal to attend to randomly presented visual stimuli within a short period of time in order to receive reinforcement. Deficits in attention can be detected by failures to respond, increased latencies to respond, and incorrect responses. Performance on this task can be altered by making the task more difficult by shortening the length of time the visual stimulus is presented and by presenting a brief burst of white noise at the time of stimulus onset.

The purpose of this study was to model the cognitive impairment that accompanies chemotherapy. Taxol, an increasingly popular chemotherapeutic agent was

used. Taxol is an antineoplastic that exerts effects on tumors and metastatic particles by creating intracellular hyperstabilization of microtubulin B. This results in an inability of the cell to undergo mitosis, leading to apoptosis of cells (Dina et al, 2001). Further, Taxol has an antiangiogenic effect, which assists in killing tumors and cancer cells (Lennernas et al, 2003). This drug, unlike many other chemotherapeutic agents, does not produce significant increases in sickness behavior and weight loss (Authier et al, 2000; Polomano et al, 2001; Weng et al, 2005).

Taxol reliably produces peripheral neuropathy (Authier et al, 2000; Peters et al, 2007; Postma et al, 1995; Polomono et al, 2001). Weng et al (2005) showed that this neuropathy may be due in part to a down regulation of glial glutamate transporters. This down regulation leads to impaired glutamate reuptake and an increase in spontaneous activity in dorsal horn neurons. This ultimately leads to a state of hyperalgesia. For this study, Taxol treated animals were expected to exhibit signs of peripheral neuropathy.

Due to the hypothesized role of cytokines in chemotherapy related cognitive impairment, measures of IL-6 and IL-1β were taken in addition to measures of neuropathy and cognitive impairment. It was hypothesized that animals receiving Taxol would show decrements in performance on the 5CSRTT relative to control animals and that these decrements would be accompanied by signs of peripheral neuropathy and heightened levels of the cytokines IL-6 and IL-1β.

CHAPTER 2

METHODS

2.1 Subjects

Twenty adult male Long Evans rats were used for this study. Ten animals were treated with Taxol and ten animals received vehicle. All animals were housed singly due to the excretion of Taxol in urine and feces in a temperature controlled room on a 12:12 (7am to 7pm) light/dark cycle with free access to water. Due to the use of operant procedures, animals were food deprived to 80 to 85 % ad libitum weight. All procedures were approved by the Institutional Animal Care and Use Committee for the University of Texas at Arlington and further adhered to the guidelines set forth by the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Zimmerman, 1983).

2.2 The Five Choice Serial Reaction Time Task (5CSRTT)

All operant conditioning occurred in an 8x11x12 inch Plexiglas chamber (Med Associates, Vermont, USA). The floor of the chamber is made of steel bars that are each 3/16 inches wide. Centered on one end of the chamber is a food hopper from where animals retrieve earned 45 mg pellets. A white light ("house light") located just above the food hopper provides illumination in the chamber throughout testing. On the wall opposite from the food hopper is a panel that contains 5 nose poke holes, each of which

has a small light located within. The entire chamber is placed inside a fan-exhausted isolation chest to ensure a consistent testing environment.

On day one of testing, animals were magazine trained for 15 minutes. On the following day, animals were exposed to the 5CSRTT. For this task, a light in one of the 5 nose poke holes is randomly presented. The length of time the light is on, which is referred to as the stimulus duration (SD), can be varied by the experimenter. From the time the light extinguishes, the animal has only a set amount of time to respond to the light by placing its nose inside the hole. This time frame is referred to as the limited hold, and is set at 5 seconds for this experiment. If the animal correctly chooses the lit nose poke hole during this time, a pellet is dispensed. If the animal responds incorrectly or fails to make a response (omission), the house light is turned off for a 5 second time-out period. The time from making a response, or failing to make a response, to reward collection is considered one trial. Each animal was tested daily for one session, which is considered to be the shorter of 100 trials or 30 minutes. Throughout training, animals were gradually moved from a 60 second SD to a 0.5 second SD as performance improved. To be included in the study animals had to maintain a criterion of 70% correct and fewer than 20% omissions for 3 consecutive sessions. Once an animal achieved criterion, the chemotherapeutic agent was administered. On the eighth day following inclusion into the study, a noise paradigm was included in the regular 5CSRTT program. For this test day only, a brief 80dB burst of white noise was randomly presented at the time of stimulus onset for approximately twenty percent of all trials. At the end of a session the percentage of correct responses, the percent of

omissions, the percentage of intertrial interval (ITI) responses (a measure of impulsivity), the average latency to make a correct response, the average latency to make an incorrect response, and the average latency to collect an earned reward were calculated according to the following formulas:

Percent correct= (Correct responses) / (Correct responses + Incorrect responses)

Percent omission= (Number of trials in which the animal failed to make a response) / (Sum of all trials for that session)

Percent ITI= (Number of trials in which to animal made a response during the intertrial interval when no stimulus is being presented) / (Correct responses + Incorrect responses + Number of ITI responses)

Latency measures= (Total latency for any given measure for the entire session) / (Total number of trials for that session)

2.3 Mechanical Paw Withdrawal Threshold

To test for the development of peripheral neuropathy, animals were tested for changes in mechanical paw withdrawal threshold (MPWT) values every other day for the duration of the 20 day protocol (Baseline and Days 1, 3, 5, 7, 9, 11, 13, 15, 17, and 19). For this test, animals were placed within a Plexiglas chamber (20X10.5X 40.5 cm) and allowed to habituate for 15 min. The chamber was positioned on top of a mesh screen so that mechanical stimuli could be administered to the plantar surface of both hindpaws. Mechanical threshold measurements for each hindpaw were obtained using the up/down method (Dixon, 1980) with eight von Frey monofilaments (3.91, 5.91, 9.97, 19.81, 38.82, 78.14, 141.99, and 239.04 mN). Each trial began with a von Frey

force of 9.97 mN delivered to the right hindpaw for approximately 1 second, and then the left hindpaw. If there was no withdrawal response, the next higher force was delivered. If a response was made, the next lower force was delivered. This procedure continued until no response was made at the highest force (239.04 mN) or until four stimuli were administered following the initial response. The withdrawal threshold for each paw was calculated using the following formula: [Xth]log=[vFr]log + ky, where [vFr] is the force of the last von Frey used, k =0.2492 which is the average interval (in log units) between the von Frey monofilaments, and y is a value that depends upon the pattern of withdrawal responses. If an animal did not respond to the highest von Frey hair, then y =1.00 and the mechanical paw withdrawal response for that paw was calculated to be 424.30 mN. The MPWT testing was performed across three trials per session and the withdrawal values were averaged over the three trials to determine the mean mechanical paw withdrawal threshold for the right and left paw for each animal.

2.4 Measurement of cytokine levels

Three times during the protocol (Baseline and Days 13 and 19), serum was collected for cytokine level analysis immediately following MPWT measurement. The baseline level collection occurred just prior to the first injection of chemotherapy. Animals were anesthetized with isoflurane, and .4 to .5 mL of blood was taken from the ventral tail vein. Blood samples were allowed to clot for approximately 45 minutes before centrifuging for 15 minutes at 1000 x g. Serum was immediately aliquoted and stored at -80 degrees C until later ELISA testing. Cytokine levels were then determined

using the quantitative enzyme immunoassay technique (R&D Systems, Minnesota, USA).

2.5 Drugs

Taxol (Sigma-Aldrich, USA) was dissolved into a 1:1 mixture of Cremaphore EL and ethanol to make a 10 mg/ml stock solution. On injection days, the stock solution was diluted to a 1 mg/ml solution with normal saline and given i.p. at 1 mg/kg. Animals in the control group received equivalent volumes and doses of the vehicle solution.

2.6 Procedures

Once animals reached the criteria of 70% correct and less that 20% omission for three consecutive days on the 5CSRTT, a baseline measure (Day 0) of MPWT was taken. Immediately following this measure, blood was collected and animals were injected with Taxol. MPWT measurements were then taken on days 1, 3, 5, 7, 9, 11, 13, 15, 17, and 19 immediately following completion of the 5CSRTT. On days 2, 4, 6, 8, 10, and 12 animals were injected with Taxol one hour following completion of the 5CSRTT. In total, animals were given 7 injections of Taxol or vehicle. Blood was collected on days 13 and 19 immediately following MPWT measurement. The result is that animals were tested daily in the 5CSRTT, Taxol injections and MPWT measurements occurred on alternating days, and blood collection occurred midway thorough and terminally in the protocol.

2.7 Data analysis

A repeated measures mixed ANOVA was used for statistical analyses on the operant and MPWT data. Drug (Taxol, Vehicle) and Time served as the independent

variables for each of the dependent variables. A .05 significance level was used. Post hoc analyses were performed using the Tukey test. For all operant measures, the raw data was analyzed followed by an analysis of normalization of the raw data. The data were normalized according to the following formula: (Day of interest – Baseline) / (Baseline) * 100. Cytokine data were analyzed with separate independent t tests for each time period (Baseline, Day 13, and Day 19) due to missing data points.

CHAPTER 3

RESULTS

3.1 MPWT results

The analysis for MPWT values yielded a significant main effect for Drug ($F(1, 18) = 429.65$; $p<.01$) and Time ($F(10, 180) = 18.55$; $p<.01$) in addition to a significant Drug X Time interaction ($F(10, 180) = 19.71$; $p<.01$). Post hoc analysis indicated Taxol treated animals exhibited significantly lower threshold values by Day 1 ($p<.01$), with maximal differences seen by Day 9 ($p<.01$) (Figure 1).

3.2 Overall 5CSRTT results

The analysis for percent correct did not indicate a significant main effect for Drug ($F(1, 18) = .62$; $p>.05$) or Time ($F(19, 342) = .86$; $p>.05$) or a significant Time X Drug interaction ($F(19, 342) = .89$; $p>.05$) (Figure 2a). Analyses of the normalized data did not indicate a significant main effect for Drug ($F(1, 18) = 1.82$; $p=.19$) or Time ($F(18, 324) = .60$; $p>.05$) or a significant Drug x Time interaction ($F(18, 324) = .81$; $p>.05$) (Figure 2b). A power analysis conducted on the normalized data for Drug indicated power was at 99%. Overall, there were no decrements in percent correct for Taxol treated animals relative to vehicle treated animals.

The analysis for percent omission did not indicate a significant main effect for Drug ($F(1, 18) = 1.20$; $p>.05$) or Time ($F(19, 342) = .99$; $p>.05$) or a significant Time X Drug interaction ($F(19, 342) = .31$; $p>.05$) (Figure 3a). Analyses of the normalized

data did not indicate a significant main effect for Drug ($F(1, 18) = .08$; $p>.05$) or Time ($F(18, 324) = 1.02$; $p>.05$) or a significant Drug x Time interaction ($F(18, 324) = .40$; $p>.05$) (Figure 3b). These results indicate Taxol treated animals were not making fewer responses than vehicle treated animals.

The analysis for percentage of intertrial interval responses did not indicate a significant main effect for Drug ($F(1, 18) = .45$; $p>.05$) or Time ($F(19, 342) = 1.01$; $p>.05$) or a significant Time X Drug interaction ($F(19, 342) = 1.15$; $p>.05$) (Figure 4a). Analyses of the normalized data did not indicate a significant main effect for Drug ($F(1, 18) = .23$; $p>.05$) or Time ($F(18, 324) = .52$; $p>.05$) or a significant Drug x Time interaction ($F(18, 324) = 1.17$; $p>.05$) (Figure 4b). Taxol treated animals were not more impulsive than vehicle treated animals.

The analysis for the latency to make a correct response did not indicate a significant main effect for Drug ($F(1, 18) = .77$; $p>.05$) or Time ($F(19, 342) = 1.59$; $p>.05$) or a significant Time X Drug interaction ($F(19, 342) = 1.24$; $p>.05$) (Figure 5a). Analyses of the normalized data did indicate a significant main effect for Time ($F(18, 324) = 1.66$; $p<.05$) but did not indicate a significant main effect for Drug ($F(1, 18) = 2.71$; $p>.05$) or a significant Drug x Time interaction ($F(18, 324) = 1.21$; $p>.05$) (Figure 5b). These results indicate it did not take Taxol treated animals longer to make a correct response relative to vehicle treated animals.

The analysis for latency to make an incorrect response did not indicate a significant main effect for Drug ($F(1, 18) = 1.05$; $p>.05$) or Time ($F(19, 342) = 1.24$; $p>.05$) or a significant Time X Drug interaction ($F(19, 342) = .65$; $p>.05$) (Figure 6a).

Analyses of the normalized data did not indicate a significant main effect for Drug ($F(1, 18) = .03$; $p>.05$) or Time ($F(18, 324) = 1.41$; $p>.05$) or a significant Drug x Time interaction ($F(18, 324) = .66$; $p>.05$) (Figure 6b). There were no differences between Taxol and vehicle treated animals in the time it took to make an incorrect response.

The analysis for latency to collect earned rewards did not indicate a significant main effect for Drug ($F(1, 18) = 2.92$; $p=.10$) or Time ($F(19, 342) = .92$; $p>.05$) or a significant Time X Drug interaction ($F(19, 342) = .99$; $p>.05$) (Figure 7a). A power analysis indicated there was sufficient power to detect a group difference (power = 99.2%). Analyses of the normalized data did not indicate a significant main effect for Drug ($F(1, 18) = 1.67$; $p>.05$) or Time ($F(18, 324) = .78$; $p>.05$) or a significant Drug x Time interaction ($F(18, 324) = .90$; $p>.05$) (Figure 7b). There were no differences between Taxol and vehicle treated animals in the time it took to collect earned rewards.

3.3 Impact of noise in the 5CSRTT

To isolate the effects of adding the noise paradigm on day 8, a repeated measures mixed ANOVA was conducted for each of the operant variables with day 8 excluded from the analysis. A 2 way ANOVA was then conducted on days 7 and 8, with day 7 serving as a posttest measure and day 8 serving as a test day. These analyses were then repeated for all normalized data.

The analysis for percent correct did not indicate a significant main effect for Drug ($F(1, 18) = .52$, $p>.05$), Time ($F(18, 324) = .69$, $p>.05$), or the Time x Drug interaction ($F(18, 324) = .86$, $p>.05$). The 2 way ANOVA also did not reveal a significant main effect for Drug ($F(1, 18) = .71$, $p>.05$), Time ($F(1, 18) = 4.34$,

p=.052), or the Time x Drug interaction (F (1, 18) = 2.34, p>.05). The analyses for the normalized data did not indicate a significant main effect for Drug (F (1, 18) = 1.66, p>.05), Time (F (17, 306) = .44, p>.05), or the Time x Drug interaction (F (17, 306) = .80, p>.05). The 2 way ANOVA for days 7 and 8 did not reveal a significant main effect for Drug (F (1, 18) = 1.74, p>.05), Time (F (1, 18) = 4.01, p>.05), or the Time x Drug interaction (F (1, 18) = 2.13, p>.05). These data indicate that the percentage of correct responses was not significantly altered for Taxol treated animals by adding the noise paradigm on day 8.

The analysis for percent omission did not indicate a significant main effect for Drug (F (1, 18) = 1.19, p>.05), Time (F (18, 324) = 1.00, p>.05), or the Time x Drug interaction (F (18, 324) = .31, p>.05). The 2 way ANOVA also did not reveal a significant main effect for Drug (F (1, 18) = 1.52, p>.05), Time (F (1, 18) = .68, p>.05), or the Time x Drug interaction (F (1, 18) = .72, p>.05). The analyses for the normalized data did not indicate a significant main effect for Drug (F (1, 18) = .09, p>.05), Time (F (17, 306) = 1.03, p>.05), or the Time x Drug interaction (F (17, 306) = .43, p>.05). The 2 way ANOVA for days 7 and 8 did not reveal a significant main effect for Drug (F (1, 18) = .09, p>.05), Time (F (1, 18) = 2.02, p>.05), or the Time x Drug interaction (F (1, 18) = .18, p>.05). These data indicate that the percentage of omitted trials was not significantly altered for Taxol treated animals by adding the noise paradigm on day 8.

The analysis for intertrial interval responses did not indicate a significant main effect for Drug (F (1, 18) = .38, p>.05), Time (F (18, 324) = .95, p>.05), or the Time x Drug interaction (F (18, 324) = 1.18, p>.05). The 2 way ANOVA also did not reveal a

significant main effect for Drug ($F(1, 18) = 2.04$, $p>.05$), Time ($F(1, 18) = 1.02$, $p>.05$), or the Time x Drug interaction ($F(1, 18) = .64$, $p>.05$). The analyses for the normalized data did not indicate a significant main effect for Drug ($F(1, 18) = .33$, $p>.05$), Time ($F(17, 306) = .53$, $p>.05$), or the Time x Drug interaction ($F(17, 306) = 1.09$, $p>.05$). The 2 way ANOVA for days 7 and 8 did not reveal a significant main effect for Drug ($F(1, 18) = 1.51$, $p>.05$), Time ($F(1, 18) = .80$, $p>.05$), or the Time x Drug interaction ($F(1, 18) = .08$, $p>.05$). These data indicate that the noise paradigm did not alter the level of impulsivity for Taxol treated animals relative to vehicle treated animals.

The analysis for latency to make a correct response did not indicate a significant main effect for Drug ($F(1, 18) = .71$, $p>.05$), Time ($F(18, 324) = 1.41$, $p>.05$), or the Time x Drug interaction ($F(18, 324) = 1.26$, $p>.05$). The 2 way ANOVA also did not reveal a significant main effect for Drug ($F(1, 18) = 1.41$, $p>.05$), Time ($F(1, 18) = 1.66$, $p>.05$), or the Time x Drug interaction ($F(1, 18) = .28$, $p>.05$). The analyses for the normalized data did not indicate a significant main effect for Drug ($F(1, 18) = 2.80$, $p>.05$), Time ($F(17, 306) = 1.46$, $p>.05$), or the Time x Drug interaction ($F(17, 306) = 1.23$, $p>.05$). The 2 way ANOVA for days 7 and 8 did not reveal a significant main effect for Drug ($F(1, 18) = 1.08$, $p>.05$), Time ($F(1, 18) = 1.97$, $p>.05$), or the Time x Drug interaction ($F(1, 18) = .22$, $p>.05$). These data indicate that the Taxol treated animals did not take longer to make a correct response as a result of the noise paradigm.

The analysis for latency to make an incorrect response did not indicate a significant main effect for Drug ($F(1, 18) = 1.18$, $p>.05$), Time ($F(18, 324) = 1.10$,

p>.05), or the Time x Drug interaction (F (18, 324) = .63, p>.05). The 2 way ANOVA also did not reveal a significant main effect for Drug (F (1, 18) = .33, p>.05) or the Time x Drug interaction (F (1, 18) = 3.06, p>.05), but did reveal a significant main effect for Time (F (1, 18) = 4.55, p<.05). The analyses for the normalized data did not indicate a significant main effect for Drug (F (1, 18) = .07, p>.05), Time (F (17, 306) = 1.25, p>.05), or the Time x Drug interaction (F (17, 306) = .61, p>.05). The 2 way ANOVA for days 7 and 8 did not reveal a significant main effect for Drug (F (1, 18) = .03, p>.05), Time (F (1, 18) = 4.32, p= .052), or the Time x Drug interaction (F (1, 18) = 3.74, p>.05). These data indicate that the time it took Taxol treated animals to make an incorrect response as a result of the noise paradigm was not significantly different from vehicle treated animals.

The analysis for latency to collect earned rewards did not indicate a significant main effect for Drug (F (1, 18) = 3.23, p>.05), Time (F (18, 324) = .17, p>.05), or the Time x Drug interaction (F (18, 324) = .02, p>.05). The 2 way ANOVA also did not reveal a significant main effect for Drug (F (1, 18) = 2.90, p>.05), Time (F (1, 18) = .93, p>.05), or the Time x Drug interaction (F (1, 18) = 1.00, p>.05). The analyses for the normalized data did not indicate a significant main effect for Drug (F (1, 18) = 1.62, p>.05), Time (F (17, 306) = .79, p>.05), or the Time x Drug interaction (F (17, 306) = .91, p>.05). The 2 way ANOVA for days 7 and 8 did not reveal a significant main effect for Drug (F (1, 18) = 2.08, p>.05), Time (F (1, 18) = .04, p>.05), or the Time x Drug interaction (F (1, 18) = .08, p>.05). These data indicate that the Taxol treated animals did not take longer to make collect earned rewards as a result of the noise paradigm.

3.4 Cytokine results

Due to small serum volumes, not all animals were analyzed for each of the three time points. As a result, independent t tests were conducted for each time point for the two cytokines tested. For IL-6 there were no differences obtained at baseline ($t(17) = .57$; $p>.05$), day 13 ($t(17) = -.28$; $p>.05$), or day 19 ($t(17) = .17$; $p>.05$) (Figure 8). For IL-1β there were also no differences obtained at baseline ($t(10) = 1.77$; $p>.05$), day 13 ($t(17) = -.41$; $p>.05$), or day 19 ($t(17) = 1.49$; $p>.05$) (Figure 9). These results indicate Taxol treated animals did not exhibit alterations in IL-6 or IL-1β cytokine levels.

CHAPTER 4

DISCUSSION

The purpose of this study was to reproduce commonly observed cognitive deficits seen in patients receiving chemotherapy using an animal model. It was hypothesized that animals treated with the chemotherapeutic agent Taxol would exhibit deficits in the 5CSRTT. Further, Taxol treated animals were expected to develop signs of peripheral neuropathy and increased cytokine levels.

The results indicate that 1 mg/kg of Taxol administered every other day did lead to mechanical sensitivity, a finding that supports the reports of previous researchers (Authier et al, 2000; Peters et al, 2007; Postma et al, 1995; Polomono et al, 2001; Weng et al, 2005). The data from the 5CSRTT indicate that animals did not develop decrements in the ability to attend to a cognitively demanding task despite the fact that these animals displayed an increased sensitivity to mechanical stimulation. Further, IL-6 and IL-1β cytokine levels were not altered for animals receiving Taxol.

Researchers have reported cognitive deficits using various chemotherapeutic agents in animals, but the results of this literature are equivocal, even when using similar paradigms. For instance, the Morris water maze has detected chemotherapy related cognitive impairments in some studies (Siegers et al, 2007; Winocur et al, 2006) but not in others (Shors et al, 2002). The majority of the current animal literature investigating chemotherapy related cognitive impairment focuses on two general

functions: hippocampal dependent tasks, such as the Morris water maze and emotionally motivated tasks, such as conditioned avoidance and fear conditioning tasks. While these tasks are relevant for studying cognitive deficits, they may not adequately model the condition that humans experience. Humans often show decrements in attention/concentration, executive function, and speed of information processing (Tannock et al, 2004). Further, many researchers report that observed cognitive deficits are not augmented by emotional conditions such as depression or anxiety (Schagen et al, 1999; van Dam et al, 1998). In light of these issues, the 5CSRTT provides an improved method for investigating potential chemotherapy related cognitive deficits. This method allows the researcher to capture measurements of attention, executive function, and information processing speed in the absence of a negative hedonic state. However, Taxol treated animals did not differ in 5CSRTT performance relative to control animals.

There are several possible explanations for the obtained results. One possible explanation for these results is that chemotherapy may interfere with learning, or acquisition of a new task. Reiriz et al (2006) found impairment in a test of avoidance conditioning using the chemotherapeutic agent cyclophosphamide only if the chemotherapy was administered within 24 hours prior to task training. Perhaps Taxol treated animals would have shown retardation of task acquisition had the chemotherapy been given prior to an animal reaching criterion. Future research should examine the effects of Taxol during task acquisition. Patients experiencing chemobrain are often advised to engage in mentally stimulating activities to combat cognitive decrements. If

this is effective at decreasing the symptoms of chemobrain, it is also possible that Taxol treated animals were effectively overcoming the symptoms by engaging in the 5CSRTT.

Another potential explanation of these findings is that it is the cancer or a combination of the cancer and the chemotherapy, and not the chemotherapy itself that most significantly contributes to cognitive impairment. Wefel et al (2004a & b) showed that the incidence of pretreatment cognitive impairment in women with breast cancer was between 33 to 35 %. However, both articles did report that cognitive abilities continued to decline following chemotherapy treatment. Animals in this study did not have cancer so it was not possible to singly determine the effects of chemotherapy in the presence of cancer.

A potential confound to the results of this study is that the incidence of chemobrain varies widely (17 to 75%) and may depend on several factors including the length of time a person has received therapy and the combination of treatments used (Stock et al, 1995; Wefel et al, 2004a). Although the sample of 10 Taxol treated animals used here is sufficient for statistical differences to be obtained, it is possible that more Taxol treated animals were needed to detect cognitive decrements. If animals display a similar prevalence of chemotherapy related cognitive decrements to humans, it might be expected that only a small proportion of the ten tested animals would develop decrements.

Although many cognitive domains appear to be affected by chemotherapy, the decrements are reported to be subtle and many times within the normal range of

function when measured using standardized tests such as the WAIS-R Digit Span, which measures general attention, the Trail Making Test Part B, which measures executive function, and the WMS-R, which measures visual memory (Ahles et al, 2002; Poppelreuter et al, 2004; Wefel et al, 2004a and b). If cognitive deficits had been present in our animals, it is unlikely that there was a failure to detect these decrements using the 5CSRTT. This task is sensitive to measures of general attention, executive function, and visual memory. If decrements had been present, group differences would have been obtained. Instead, it is more likely that the dose of Taxol used for this study was not high enough to induce changes in cytokines or cognitive function. The dose of Taxol used for this study (1 mg/kg) was chosen because it has been shown to lead to mechanical hypersensitivity without negatively affecting body weight or feeding behavior (Dina et al, 2001; Polomano et al, 2001; Weng et al, 2005). Higher doses in rodents (16 or 32 mg/kg) are more equivalent to the 250 mg/m2 of Taxol that humans receive but lead to severe weight loss and even death (Authier et al, 2000). Therefore, to adequately study chemotherapy related cognitive impairments, researchers will have to determine an adequate and human equivalent dose of chemotherapy, and they may need to adopt methods that do not rely on appetitive drive.

IL-6 and IL-1β levels were not altered for Taxol treated animals in this study. These particular cytokines have been shown to be altered by Taxol administration in humans and in vitro (O'Brien et al, 1995; Penson et al, 2000; Rabinowitz et al, 1993; Tsvaris et al, 2002). However, alterations in other cytokines such as IL-8 and IL-10 have been reported in response to Taxol treatment (Pusztai et al, 2004), and it is

possible that these cytokines were modified in this study. Because the interest of this study was cognitive impairment, and because an abundance of literature suggests IL-6 and IL-1β play a role in this impairment, these particular cytokines were the focus of the cytokine assessment.

The analyses for cytokine levels revealed that 1 mg/kg of Taxol administered every other day did not alter either IL-6 or IL-1β in this study. However, one caveat should be mentioned. Studies investigating IL-6 generally report very low levels of this cytokine at baseline (Ando et al, 1998; Arimura et al, 1994). The results presented here show IL-6 baseline levels may have been elevated above the levels generally reported. In fact, control animals in this study exhibited a mean of 292 pg/mL for baseline IL-6 levels. It is possible that the stress induced by the anesthesia process increased IL-6 levels and that group differences were not obtained due to a failure to obtain differences above and beyond those changes induced by stress. This conclusion is not likely due to the short amount of time it took to anesthetize and collect blood from animals. This entire process took approximately 5 to 10 minutes. Ando et al (1998) did not find increases in IL-6 levels due to restraint stress until 30 minutes following initiation of restraint. Regardless, group differences were not obtained indicating that Taxol treatment did not lead to significant alterations in cytokine levels.

Finally, research suggests that hormones, especially estrogen, may contribute to chemotherapy related cognitive decrements (Ahles and Saykin, 2007; Barton and Loprinzi, 2002). Research suggests that estrogen is protective against certain cognitive impairments and that estrogen deficiency contributes to deficits in these same cognitive

domains (Jansen et al, 2005). Because chemotherapy is associated with induced menopause, it is logical to conclude that estrogen contributes to chemotherapy related cognitive impairment. However, cognitive impairments have been observed even when menopausal status was accounted for (Ahles et al, 2002; Brezden et al, 2000). Despite this finding, it would be beneficial for future animal research to use ovariectomized females to further study the role of estrogen in chemotherapy related cognitive impairments.

Expected results would have provided a major breakthrough in the study of cancer and its symptoms. Exogenous biological response modifiers, including IL-1, IFNα, and TNFα, are used as part of a regimen to treat cancer along with chemotherapy. Unfortunately, due to side effects, many patients opt for a treatment that has fewer side effects, but is not as efficient at treating cancer. An animal model would allow for the testing of potential treatments and a better understanding of the mechanisms of some side effects, including cognitive impairment. Aside from studies on the hippocampus, the current state of the literature does not fully address what areas of the brain may be involved in chemotherapy related cognitive impairment, and an animal model would allow for further elucidation of the areas involved, furthering the potential for treatment options.

APPENDIX A

FIGURES

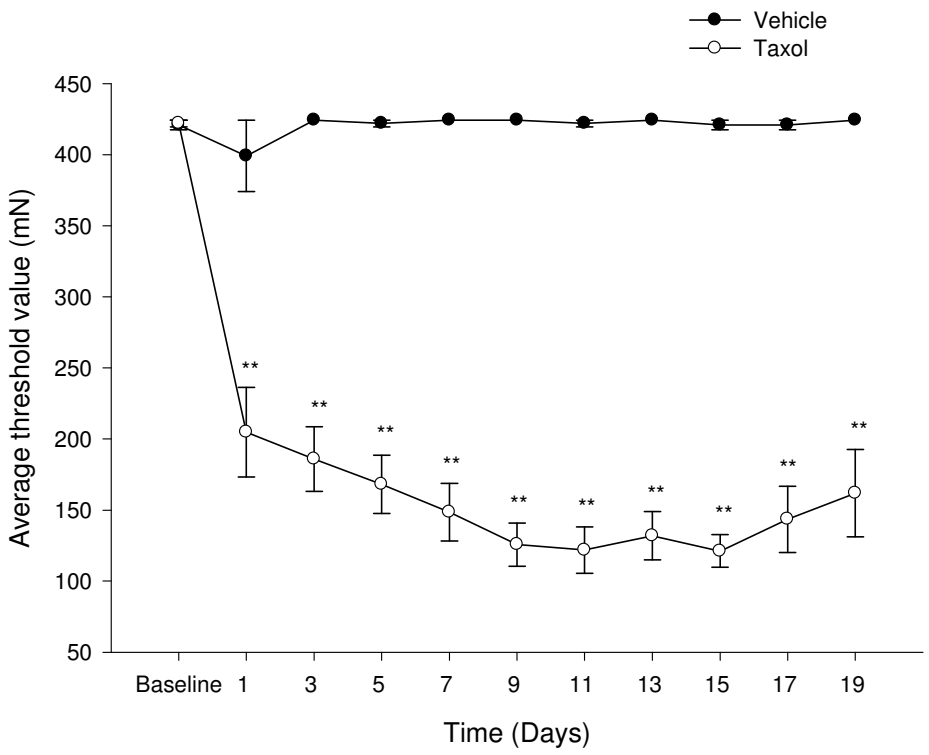

Figure 1 Mechanical Paw Withdrawal Thresholds

Mean (+/- S.E.M.) mechanical paw withdrawal threshold measurements across the test period for vehicle (n=10) and Taxol (n=10) treated animals. Animals were tested for MPWT value alterations approximately 23 hours post injection. Analysis indicated that relative to vehicle treated animals, Taxol treated animals showed decreased threshold values by Day 1, with maximum differences observed by Day 9. ** = $p<.01$

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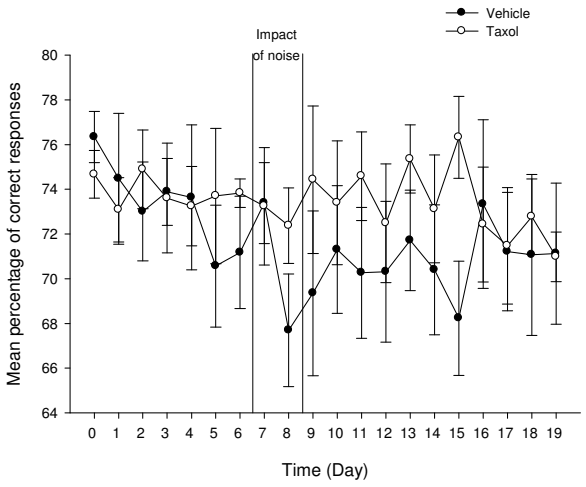

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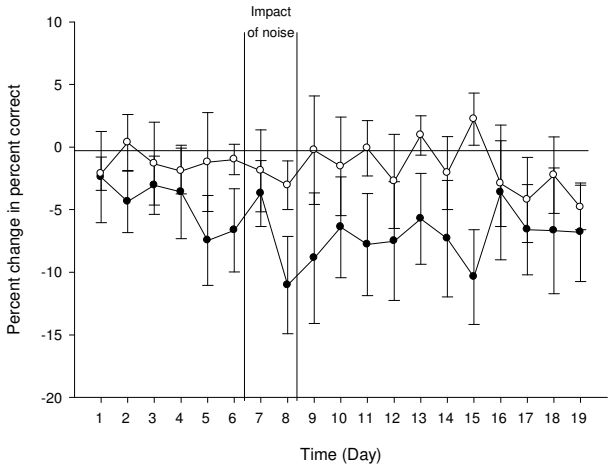

Figure 2 Percent correct in the 5CSRTT

a) Mean (+/- S.E.M.) percentage of correct responses made by animals in the 5CSRTT. Animals were tested every 24 hours in the 5CSRTT. On day 8, a burst of 80 dB white noise was randomly presented at stimulus onset. Analyses revealed no significant differences between the Taxol and vehicle treated animals for the percentage of correct responses, indicating that Taxol did not significantly alter the ability to correctly attend and respond to the visual stimuli. b) Normalized means (+/- S.E.M.) for the percentage of correct responses made by animals in the 5CSRTT. Data were normalized for each of the operant measures according to the following formula: (Day of interest – Baseline) / (Baseline) * 100. Despite the appearance that Taxol treated animals were more correct than vehicle treated animals, analyses indicated no significant differences between the Taxol and vehicle treated animals.

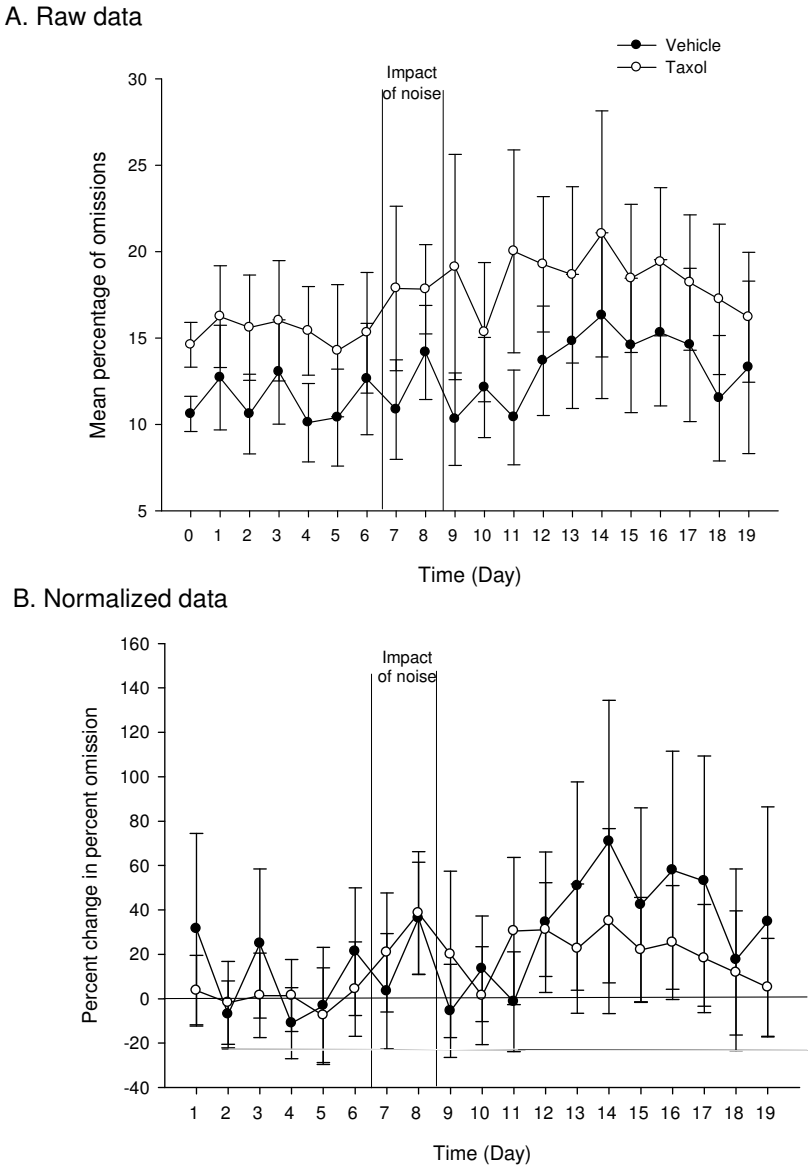

Figure 3 Percent omission in the 5CSRTT

a) Mean (+/- S.E.M.) percentage of omitted trials for animals in the 5CSRTT. Analyses indicated that, relative to vehicle treated animals, Taxol treated animals did not exhibit changes in the percentage of omitted trials. Further, the introduction of noise on day 8 did not alter performance for Taxol treated animals. b) Normalized means (+/- S.E.M.) for the percentage of omitted trials made by animals in the 5CSRTT. Analysis indicated no significant differences between the Taxol and vehicle treated animals. Introduction of noise on day 8 did not alter the percentage of trials that animals failed to respond to.

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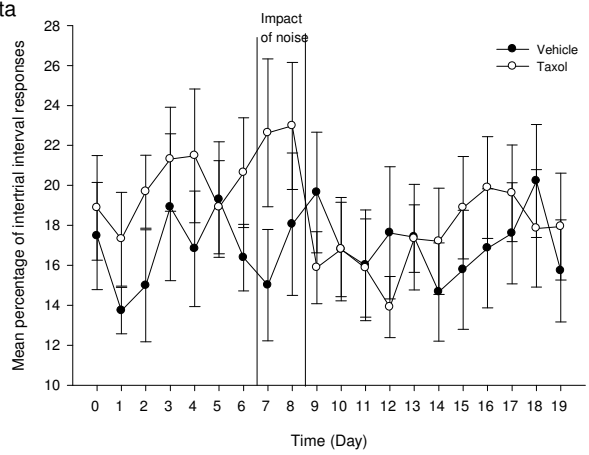

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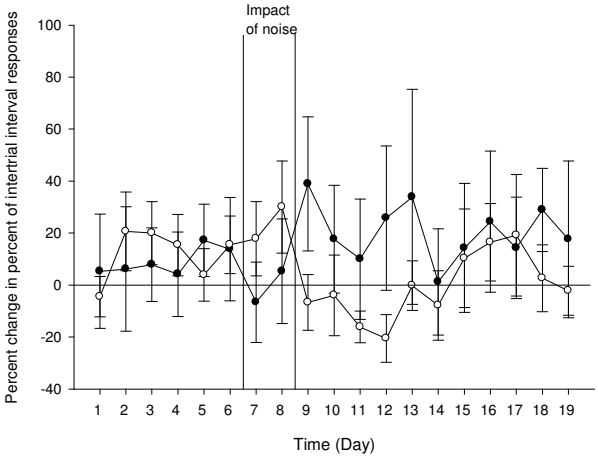

Figure 4 Percentage of intertrial interval responses

a) Mean (+/- S.E.M.) percentage of responses made during the intertrial interval for animals in the 5CSRTT, which is a measure of impulsive behavior. Analyses indicated no significant differences between the Taxol and vehicle treated animals, even when noise was introduced. These data indicate Taxol treated animals were not behaving any more or less impulsive than vehicle treated animals. b) Normalized means (+/- S.E.M.) for the percentage of responses made during the intertrial interval for animals in the 5CSRTT. Analyses indicated that Taxol treated animals were not more impulsive than vehicle treated animals.

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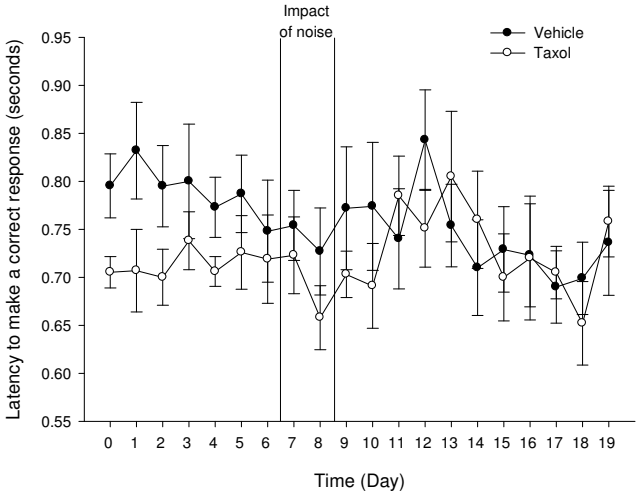

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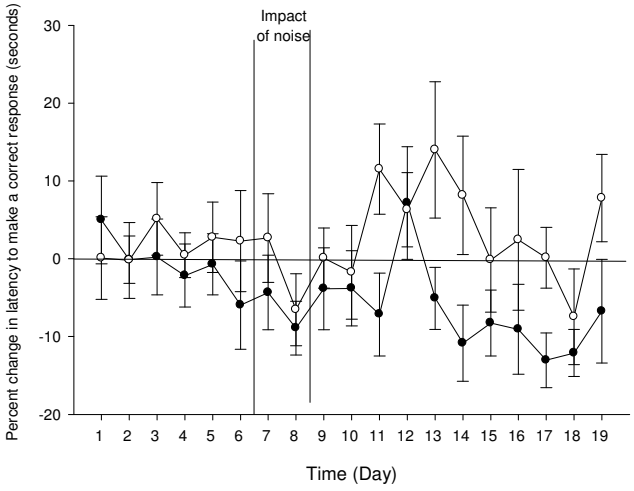
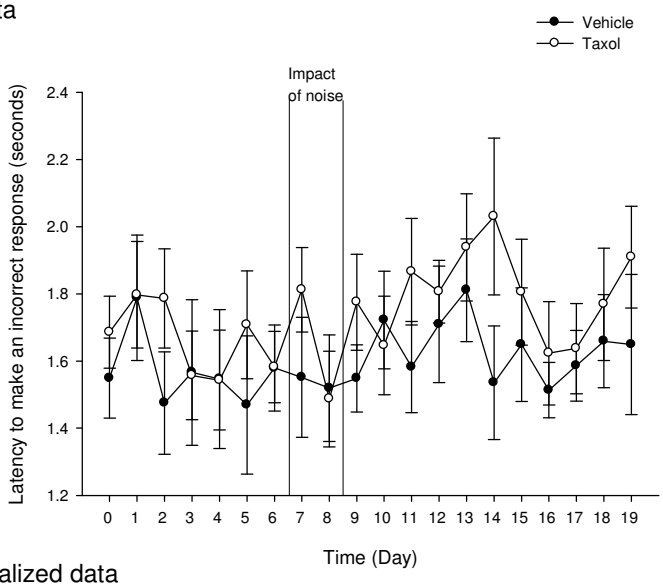


Figure 5 Latency to make a correct response

a) Mean (+/- S.E.M.) latencies to make a correct response for animals in the 5CSRTT. Analyses revealed that relative to vehicle treated animals, Taxol treated animals did not take longer to make a correct response. A lack of modification to latency data in combination with a lack of changes to other data indicates motivation and attention are unaltered in the task. b) Normalized means (+/- S.E.M.) for the latencies to make correct responses for animals in the 5CSRTT. Analyses show that Taxol treated animals did not take longer to make a correct response than vehicle treated animals.

A. Raw data



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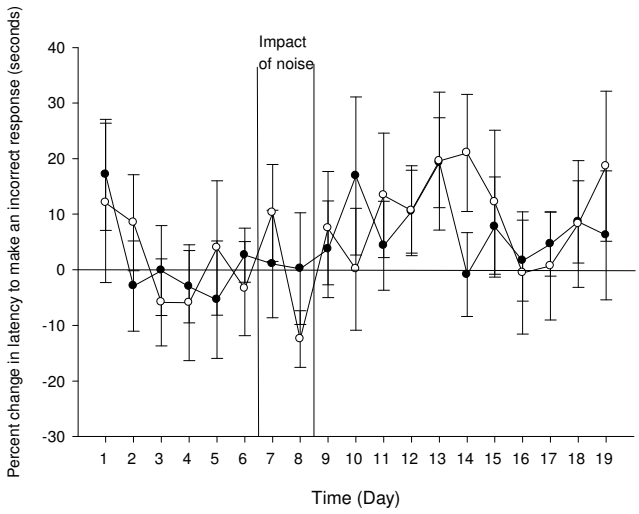
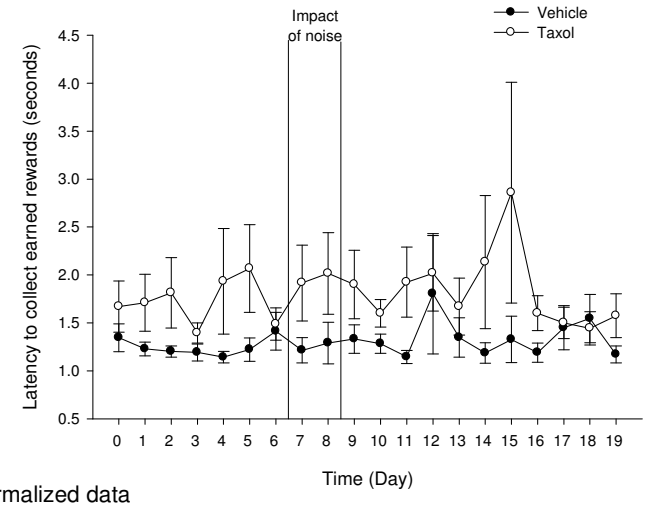


Figure 6 Latency to make an incorrect response

a) Mean (+/- S.E.M.) latencies to make an incorrect response for animals in the 5CSRTT. Analyses revealed no significant differences between the Taxol and vehicle treated animals, indicating Taxol treated animals did not take more or less time than vehicle treated animals to make an incorrect response. b) Normalized means (+/- S.E.M.) for the latencies to make incorrect responses for animals in the 5CSRTT. Analysis revealed no significant differences between the Taxol and vehicle treated animals, indicating Taxol treated animals did not take longer, or less time, to make an incorrect response.

A. Raw data



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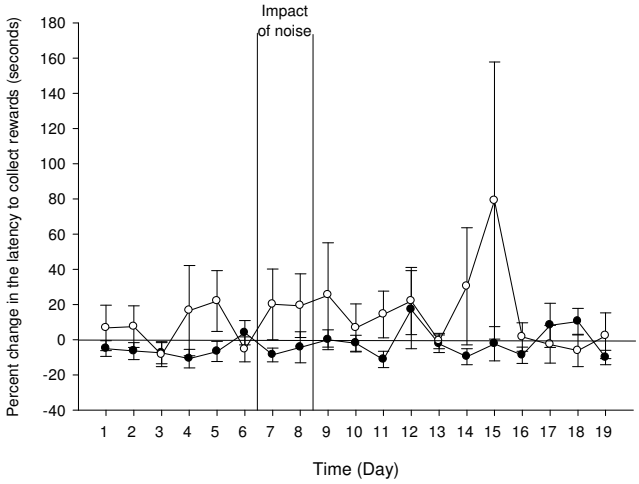

Figure 7 Latency to collect earned rewards

a) Mean (+/- S.E.M.) latencies to collect an earned reward for animals in the 5CSRTT. Analyses indicated Taxol treated animals were not significantly different in the amount of time it took to retrieve earned food pellets. b) Normalized means (+/- S.E.M.) for latencies to collect earned rewards for animals in the 5CSRTT. Although it appears that Taxol treated animals took longer to collect food pellets, analyses indicated no significant differences between the Taxol and vehicle treated animals, even with the introduction of noise on day 8.

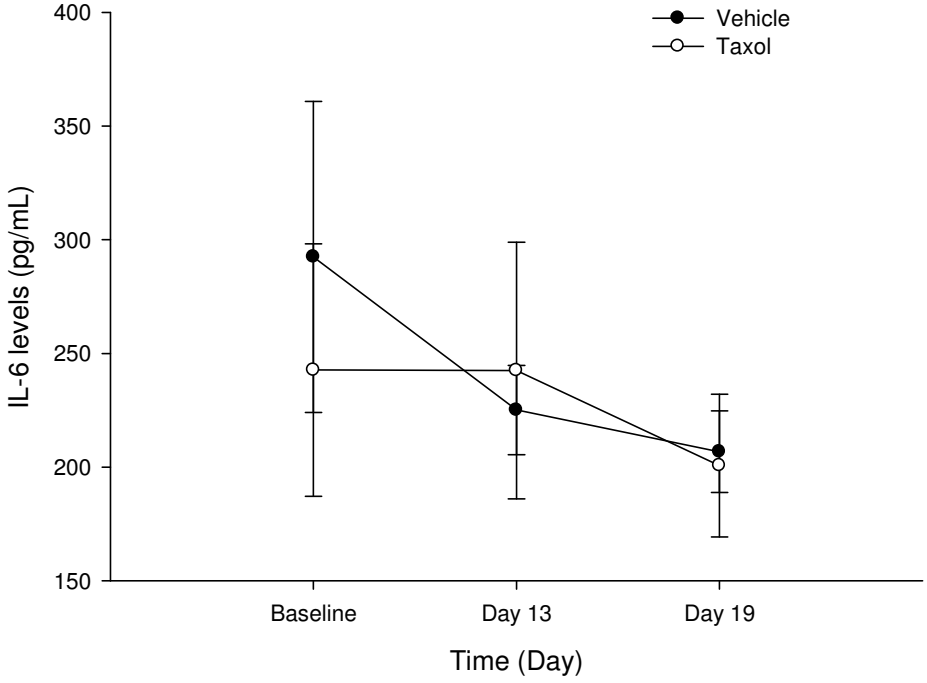

Figure 8 IL-6 cytokine levels

Means (+/- S.E.M.) for IL-6 serum levels across the test period. Blood was collected immediately following MPWT measurements. Analyses indicated no significant differences between Taxol and vehicle treated animals, indicating Taxol treatment at 1 mg/kg every other day does not increase IL-6 levels.

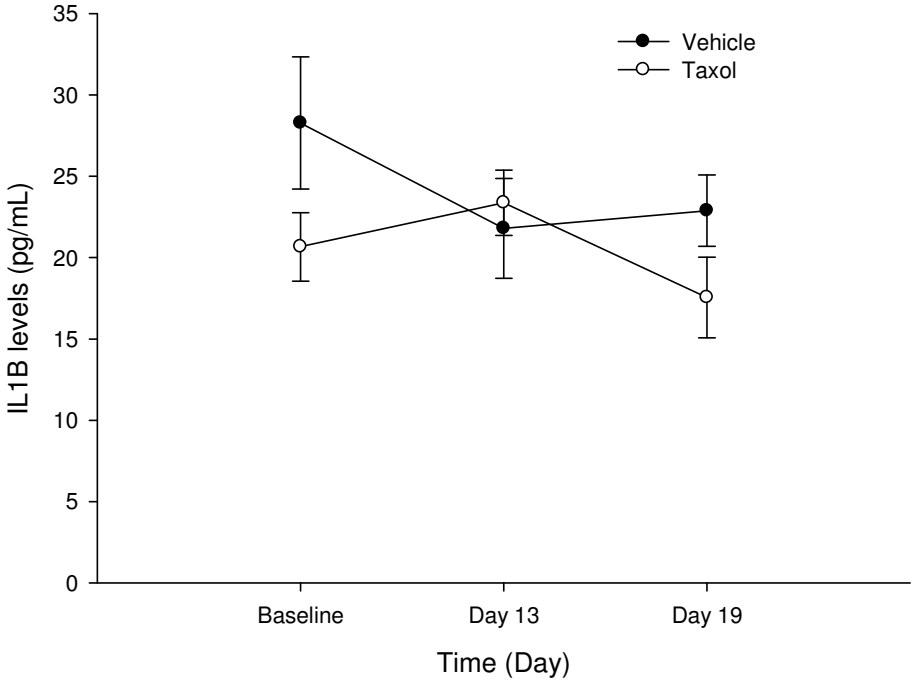

Figure 9 IL-1β cytokine levels

Means (+/- S.E.M.) for IL-1β serum levels across the test period indicated no significant differences between Taxol and vehicle treated animals. These data indicate that 1 mg/kg Taxol given every other day does not increase IL-1β levels beyond those of control animals.

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BIOGRAPHICAL INFORMATION

Jessica A Boyette-Davis was born in Fort Worth, Texas and grew up in central Texas. She graduated from Tarleton State University with a Bachelor of Science degree in Psychology in 2001, and began attending the University of Texas at Arlington in August 2005. While here, she has conducted a variety of translational experiments investigating issues such as schizophrenia, depression, and peripheral neuropathy. She has also investigated various aspects of attention using animal models. In the future, she hopes to continue this line of research in an academic setting.