THE NEUROCOGNITIVE EFFECTS OF VANADIUM IN YOUNG MALE RATS

by

Amanda J. Dyer

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ABSTRACT

Previous research on vanadium has shown evidence of toxicity when animals and humans are exposed. Vanadium has negative physiological consequences when administered such as respiratory and gastrointestinal problems. However, in the area of diabetes research, vanadium has shown benefits in treatment, such as control of blood sugar, and reducing the need for insulin. Others have shown both negative and positive cognitive effects, which necessitates the need for more information about vanadium exposure. The current study investigated the effects of vanadium exposure (0.05 mg/1000 mg of food mash) on neurocognition in rats. Four weeks following administration of vanadium, rats were tested on the Open-Field, Object Recognition, and Morris Water Maze tasks. Vanadium exposure did not yield significant results on the Open Field test, or on the Object Recognition Task. However, vanadium exposure did improve spatial memory on day 2 of the Morris Water Maze, and there was a trend on days 3 and 4. This study indicates vanadium may have a positive impact on cognition, warranting further research to understand more about the benefits and consequences of vanadium administration.

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Approved:

_____ _____
Chairman, Thesis Committee Date

_____ _____
Member, Thesis Committee Date

_____ _____
Member, Thesis Committee Date

_____ _____
Head, Major Department Date

_____ _____
Dean, Graduate School Date

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CHAPTER I

INTRODUCTION

Environmental exposure to heavy metals is a public health hazard that has been linked to cognitive and neurological deficits. Exposure to heavy metals can occur through contaminated food, water, air, or in industrial work settings. To date, the majority of research investigating the deleterious effects of environmental toxins on neurocognition has focused on heavy metals such as lead and mercury (Liu & Lewis, 2014). For instance, exposure to lead through the diet or as a result of working in an industrial setting, has been found to be associated with deficits in attention, processing speed, visuospatial abilities, working memory, and motor functioning (Seo et al., 2014). The reason for this is unknown, and more research is needed in order to better understand the neurological changes associated with these findings.

In addition, the neurotoxic effects of various transition metals such as nickel, cadmium, and chromium have also been studied. Chronic exposure to these metals has been found to result in a variety of negative health effects from difficulties in concentration and balance to cancer (Das, Das, & Dhundasi, 2008; Vianene et al., 2000).

Of increasing interest in recent years are the health risks and/or benefits of exposure to the transition metal vanadium. This metal is present in the natural environment and is used in industry for machine and tool making. For example, in a study on humans, researchers found better performance on memory and learning tasks

when exposed to a low level of vanadium through drinking water, posing the question of benefits of exposure. To date, much of the research on vanadium has focused on areas concerning respiratory effects, diabetes, mental health, and toxicology of those exposed. Research has shown both positive and negative effects of vanadium exposure on animals and humans. To date, knowledge is lacking on the neurocognitive effects of vanadium exposure. Hence, the current study will examine the physiological consequences of vanadium ingestion as well as add to the current literature by examining the neurocognitive consequences of chronic vanadium ingestion in young male rats.

CHAPTER II

LITERATURE REVIEW

Vanadium

Vanadium (V) is an essential trace element at position 23 on the periodic table and is a metal. Because vanadium is hard, corrosion-resistant, and readily forms alloys when combined with other metals, its use is popular in industrial settings (Barceloux, 1999). Production of vanadium in the 1970's was approximately 35,000 tons throughout the world. Uses of vanadium are predominately in industrial settings, as they make frequent use of vanadium as a catalyst in the production of sulfuric acid and in converting organic compounds into plastic. Further uses include: semiconductor manufacturing, photo development, creating artificial yellow pigments, and in the production of ceramics (Barceloux, 1999).

Human studies on vanadium have indicated a number of environmental effects this metal may potentially have on health. For example, in the general population, vanadium appears to be mostly exposed (but not limited to) to humans through food. Dietary intake of vanadium-containing food include: black pepper, dill seeds, mushrooms, parsley, shellfish, and spinach (Barceloux, 1999). Particular types of foods contain higher and lower levels of vanadium, such as seafood (a higher concentration), and vegetables (a lower concentration). Interestingly, processed foods generally contain increased amounts of vanadium compared to unprocessed foods (Barceloux, 1999).

Other exposure to vanadium ingestion includes drinking water, however there is little information regarding groundwater vanadium levels (Wright & Belitz, 2010). Although water usually does not contain a great amount of vanadium (a few micrograms per liter), some water supplies have been found to contain vanadium concentrations over 100 μg/L, which has led to some regulation of vanadium in drinking water (Crebelli & Leopardi, 2012; Wright & Belitz, 2010). Although animal research has indicated repeated vanadium exposure by drinking water is not generally a concern, high levels of vanadate in drinking water does elicit some genotoxicity (Crebelli & Leopardi, 2012). The general population ingests an average of 10-60 μg vanadium per day through the diet (Barceloux, 1999).

Physiological Effects in Humans

The most prevalent studied form of vanadium exposure is by inhalation, due to the well-known toxic symptoms observed when humans and animals are exposed to air containing vanadium. Since vanadium is a common metal used in work settings for machines and tools (Barceloux, 1999), interest has been shown concerning the influence it has on the environment and respiratory effects on individuals who have been exposed to this element.

Early reports of toxic effects from vanadium exposure were reported in the 1940's (Williams, 1952). Researchers have investigated the physiological consequences of vanadium exposure in men working in an industrial setting. Within the work environment were boilers that consumed approximately 150 tons of fuel per week, with vanadium contents approximating at 1 lb. of vanadium per 11 tons of fuel. Men who were most at risk for vanadium exposure were those who cleaned the boilers. Once a

year, eight men were required to clean the boiler, a task that took five days. This task resulted in large amounts of vanadium particles (dust) to be dispersed into the working environment (Williams, 1952).

Exposed workers complained of a variety of symptoms including: rhinorrhea, sneezing, watery eyes, dry-cough, wheezing, dyspnea (shortness of breath), soreness behind the sternum, greenish-black coloring of the tongue, and râles (abnormal rattling sound that occurs when breathing). Secondary effects were also reported, including symptoms of sleep disturbance, difficulty walking for prolonged amounts of time, difficulty climbing stairs (due to shortness of breath), vomiting (due to excessive coughing), exhaustion, weakness, and depression (Williams, 1952).

In a later study, Diamond, Caravaca, and Benchimol (1963) investigated the physiological effects of oral administration of vanadium in six male individuals. Ammonium vanadyl tartrate was administered one to four times daily in 25mg tablets. Length of treatment varied between 6-9 weeks among subjects. Total vanadium amounts also varied among the men ranging from 3450mg to 4325mg. Of interest was to determine if vanadium tolerance would be established, if there was a relationship between dosage and excretion, if there would be toxic effects, and to elucidate its effect on circulating lipids (Diamond et al., 1963). Some individuals yielded unpredictable excretory results, and all participants showed a variation of vanadium amounts in urinary excretion. Although laboratory results suggested no toxicity of the blood, complaints from participants suggested toxic effects. Participants reported black loosened stools, stomach cramps, and diarrhea (Diamond et al., 1963).

This study, like others, reported short periods of time where discoloration of the tongue was present, however, this study pinpointed a dosage (starting at 50 mg), where the tongue discoloration began (Barceloux, 1999; Diamond et al., 1963; Williams, 1953). Others have self-reported fatigue, lethargy, and a few participants reported dysmenorrhea (intense menstrual cramps), while taking vanadium tablets. Researchers found that coadministration of triglyclamol chloride at doses of 50 mg with vanadium tablets diminished the reported intestinal symptoms (Diamond et al., 1963).

Large variations in excretion imply that there is an unpredictable absorption of vanadium with the oral administration method. Additionally, reports from participants about gastrointestinal difficulties could suggest overdose. Interestingly, the coadministration of triglyclamol chloride, which is a cholinergic-blocking drug, suggests that vanadium may have an influence on the cholinergic system (Diamond et al., 1963). These early studies were important in determining the toxicological consequences of exposure to high levels of vanadium. Research has continued throughout the past several decades to further understand the consequences of exposure to this metal.

A recent review revealed that there should be an establishment of an occupational exposure limit (OEL) in regards to vanadium for industrial workers and other occupational settings that expose workers to vanadium (Assem & Levy, 2009). Vanadium has been a difficult metal to establish an OEL, due to the differences in OEL-setting committee's interpretations of research results that indicate toxicity of vanadium. For example, the German MAK Commission finds that all vanadium compounds should be considered carcinogenic due to structure and interconversion of ionic vanadium species, while the U.S. American Conference of Governmental Industrial Hygenists

(ACGIH) based its decision on vanadium toxicity from the chronic upper respiratory effects it has on exposed workers. The ACGIH's threshold level value (TLV) on vanadium aims to protect the risk of lung cancer in individuals.

In order to establish an OEL, committees must look at toxicological research that has been done on vanadium. The National Toxicology Program (NTP) conducted a toxicological study that is most commonly used by committees who establish an OEL for vanadium in work settings. The NTP study was done on mice and rats administered vanadium pentoxide (V_2O_5). Researchers concluded that there was evidence of lung tumors in both male and female mice, and when using rats, there was evidence of carcinogenicity in males. Furthermore, all V_2O_5 exposed mice had significantly more occurrence of alveolar/bronchiolar adenomas and carcinomas, but there was no dose-response relationship. In rats, alveolar/bronchiolar adenomas and carcinomas were found in males, both in controls and those exposed to vanadium, and there was also no dose-response relationship. Assem and Levy (2009) concluded that the NTP study on V_2O_5 does not present enough evidence to fully understand the effects of vanadium on the body, but it is considered a local carcinogen to the lungs.

Other research has found that exposure to Vanadyl ($VOSO_4$) resulted in DNA damage. Additionally, a reduction in DNA repair of human lymphocytes was seen, which suggests there may be double strand DNA breaks in the cells (Assem & Levy, 2009; Wozniak & Blasiak, 2004). The process by which DNA damage is induced is not clear, however, a role for H_2O_2 is suggested (Assem & Levy, 2009; Capella et al., 2002).

In the general population, individuals may be exposed to vanadium through ambient air. Zhang, Chau, Lai, and Wong (2009) examined the effects of environmental

exposure to vanadium on cardiovascular and respiratory systems. One particular component of exposure to vanadium and other metals was Residual Oil Fly Ash (RFOA), produced by combustion of fuel oil that could negatively affect health of individuals (Zhang et al., 2009). Vanadium-containing fossil fuels are alleged to emit approximately 65,000 tons of the metal into the air each year (Haider et al., 1998). Boilmakers who are highly exposed to RFOA reported increased respiratory problems, inflammation to the airway, and airway obstruction. Exposure to RFOA and vanadium compounds could also result in damage of defense mechanisms that prevent airborne pathogens. Other toxicological studies have focused on the cardiovascular and cardiopulmonary effects vanadium can induce. For example, vanadium induces a decrease in spontaneous heartbeat rate, vasoconstriction, and vasodilation (Bagate, Meiring, Cassee & Borm, 2006; Zhang et al., 2009). Additional reports have found problems of the respiratory tract, rhinitis, and nasal hemorrhage. Workers also develop these symptoms from fumes containing vanadium pentoxide (V_2O_5), the most common source of vanadium (Barceloux, 1999).

Overall, research on the toxicity of vanadium has found that exposure may result in a collection of adverse health effects including increased cardiovascular and respiratory diseases, increased incidents of lung cancer, and inflammatory responses (Zhang et al., 2009).

Neurocognitive Effects of Vanadium Exposure in Humans

In addition to its toxic effects on peripheral systems, a few studies have attempted to determine whether vanadium is neurotoxic. For instance, Barth et al., (2002), examined whether a group of 49 male workers exposed to vanadium exhibited cognitive

deficits. Measured behaviors included attention, visuospatial functioning, visuomotor functioning, reaction time, short-term memory, and prefrontal functioning. The control group included workers of a steel production plant that were not exposed to vanadium. Any individuals with previous neurological or psychiatric history were excluded from the study.

Serum vanadium levels with a range from 0 to 5 μg/L and from 0 to 40 μg/L were found. Psychological tests administered included the Wisconsin Card Sorting Test (a test of executive function, concept formation, and planning ability), the Block Design Test (a test of visuospatial abilities), Choice Reaction (to measure the ability to respond to complex stimuli by pressing buttons), Simple Reaction Time (to measure visual reaction time), Digit Symbol Substitution (to measure attention and visuomotor function), and Digit Span (to measure short-term memory).

Out of the tests administered to subjects, results indicated significant differences on the Block Design Test, and the Digit Symbol Substitution. In the Block Design Test, subjects exposed to vanadium displayed a decreased ability in visuospatial skills as opposed to the control (unexposed) group. On the Digit Symbol Substitution Test, exposed workers had lower scores than unexposed workers. Although not significant, the exposed group of workers performed slightly better on the Digit Symbol test and Reaction Time test (Barth et al., 2002). In the Block Design Test, there was a significant dose-response relationship between serum and urine vanadium levels. This study suggests that occupational vanadium exposure may lead to a variety of neurobehavioral impairments including decreased visuospatial abilities as well as attention deficits (Barth et al., 2002).

In a later study, Li et al. (2013) collected neurobehavioral data on vanadium-exposed workers from a steel and iron industry in China. The control group consisted of workers not exposed to vanadium. The test battery included Simple Reaction Time, Digit Symbol, Santa Anna Dexterity, Digit Span, Benton Visual Retention, and Pursuit Aiming tasks. Additionally, auditory tests were applied.

Scores on anger-hostility, fatigue-inertia, and vigor-activity were significantly higher in individuals working at the industrial plant for more than 10 years, compared to individuals who had worked at the plant less than 10 years. Additionally, vanadium exposed workers performed significantly worse on the forward/backward Digit Span, Digit Symbol, Benton Visual Retention Tests, and correct Pursuit Aiming tests. It was observed that the amount of years spent at the facility led to an overall decline in neurobehavioral function, suggesting a slow-occurring change in cognition, and memory. Auditory tests indicated that when counting tones, exposed workers made significantly more errors than controls (Li et al., 2013).

Li et al. (2013) indicated that central nervous system functioning was affected by vanadium exposure. Furthermore, vanadium exposure was related to more negative emotions, lower coordination, decreased short-term memory, and lower reaction speeds. Additionally, workers showed lower performance on auditory memory, motion speed, cooperation, and accuracy. Workers also showed increased reaction times compared to controls, which may be associated with decreased coordination.

Vanadium Toxicity in Rodents

Because exposure levels of vanadium may differ significantly among humans, the use of rodents allows for more controlled studies to be conducted. For instance, exposure

can be precisely controlled and virtually any type of toxic effect can be measured. Specifically, Haider, Abdel-Gayoum, El-Fakhri, and Ghwarsha, (1998) administered 1.5 mg of vanadium per kg^{-1} body weight (i.p.) for 12 days and found that rats had difficulty walking and moving their hind limbs in addition to, convulsions, muscular problems, difficulty breathing, lack of coordination, inactivity, and diarrhea. Body weight was also reduced significantly by 7.7%. Weight of the brain was also significantly lower compared to the control (saline) group.

Since inhalation of vanadium has had negative effects on humans, rodent inhalation of vanadium has also been studied (Barceloux, 1999; Raabe & Al-Bayati, 1997; Williams, 1952). In one study consisting of two separate experiments, 36 young and 36 adult rats were exposed to aerosols of vanadium pentoxide (V_2O_5). In the first experiment, adult rats were exposed to an aerosol containing V_2O_5 particles, and in the second experiment, young rats were exposed to a V_2O_5 aerosol. Results indicated that when exposed to V_2O_5, lung clearance occurred in the first 24 hours following exposure in both adult and young rats. Overall, adults had more lung burden than young rats; however, there were no significant differences in the amount of vanadium in the lobes of the lungs of either adult or young rats. A higher skeletal uptake of vanadium and longer retention was found in younger compared to older rats (Raabe & Al-Bayati, 1997).

In another study, young (22 days) and adult (62 days) rats were exposed to sodium orthovanadate (Na_3VO_4) at 10 mg/kg/day for eight consecutive days via i.p. injection (de la Torre, Granero, Mayayo, Corbella, & Domingo, 1999). Among the young rats, one rat died (for unexplained reasons) but no other rats showed any adverse effects. Compared to the young rats, adult rats showed numerous physical ailments after

exposure to vanadium, including weight loss, decreased motion, weakness, bleeding of the eyes, and red-nose. Vanadium treatment also induced a reduction in blood glucose in both young and adult rats.

Moreover, vanadium levels in the kidneys of both young and adult rats was found to be approximately 1000 and 2200 times higher than the control rats, respectively. Adult rats were also found to have vanadium amounts four times higher than the amount in control rats (de la Torre et al., 1999). Physiological changes in cellular structure could also be seen when comparing control and vanadium-treated rats. Renal changes were seen following vanadium administration. In young vanadium-treated rats, a small amount of vacuolization of cells was observed, and in adult vanadium-treated rats, vacuolization of cells was pronounced, as well as evidence of necrosis, suggesting a greater impact on older rats.

Neurocognitive Effects of Vanadium Exposure in Rodents

In addition to peripheral toxicity, a few studies have investigated whether vanadium treatment affects neurocognitive functioning. In one such study, Sanchez, Colomina, and Domingo (1998), gave sodium metavanadate by gavage for eight weeks at four doses (0, 4.1, 8.2, and 16.14 mg/kg/day). Three weeks following vanadium administration, rats were tested on an open field (to measure locomotor activity) and a two-way shock avoidance paradigm (to measure avoidance learning). Results indicated a reduction of weight gain in the group with the highest dosage that reduced gradually over time (weight gain occurred, but subsequently decreased starting at the sixth week of treatment). In regards to tissue content, vanadium was found in the liver, kidneys, and muscles. As dosages increased, the amount of vanadium present in these tissues

increased. Behavioral results showed that vanadium treated rats exhibited decreased locomotion compared to non-treated rats. Vanadium administration was further found to negatively affect avoidance performance.

In a later study, Avila-Costa and colleagues (2006) investigated the consequences of vanadium inhalation on memory in 48 cluster of differentiation-1 (CD-1) mice. Mice were first trained on the Morris Water Maze and then subjected to either 0.2M V_2O_5 or deionized water via inhalation for one hour twice a week for four weeks. One week following vanadium or vehicle exposure, mice were tested on the Morris Water Maze. Vanadium treated mice exhibited significantly longer latencies compared to control mice on this task.

Additional analyses also indicated dendritic spine loss in the hippocampus in mice exposed to vanadium. Other important findings were that neurons in the hippocampus showed necrotic cell death. Because the Morris Water Maze is a hippocampal dependent task, damage to the hippocampus as a result of vanadium exposure provided an explanation as to why mice showed impaired performance on this task. This study supports evidence that vanadium inhalation may be harmful to humans and animals.

In a similar study using rats, Azami and colleagues (2011) investigated the effects of oral administration of sodium metavanadate on Morris Water Maze performance. Rats were administered sodium vanadate every day for 2 weeks (at a dose of 0, 15, 20, or 25 mg/kg/day). One day after the last day of vanadium or vehicle treatment, rats were tested on the Morris Water Maze. This study found that rats treated with sodium vanadate showed significant deficits in spatial memory as measured by this task.

Igado, Olopade, Adesida, Aina, and Farombi (2012), further explored the effects of the administration of a flowering plant, garcenia kola in conjunction with vanadium on rats. In this study, vanadium was administered via i.p. injection either alone at doses of 1.25 mg/kg, and 1.50 mg/kg for five days and also following doses of garcenia kola, taken by mouth (first five days on garcenia kola, and second five days on vanadium)

Several interesting neuropathological findings were reported by this study. Among the vanadium treated rats, degeneration of the Purkinje cell layer of the cerebellum was observed. In addition, damage to myelin tracts was also found. Furthermore, vanadium levels were high in the hippocampus. Congestion was observed in the meningeal blood vessels in the group receiving 1.50 mg/kg vanadium, and increases in lipid peroxidation (LPO) were found in the olfactory bulb. The authors reported that these findings may account for previous findings of impairments in cognition, coordination, and movement found in previous studies.

A finding from this study was that the olfactory bulb was affected. This finding suggests that exposure to vanadium may decrease the sense of smell, which may then lead to problems in the natural environment (Igado et al., 2012).

Current Study

There currently exists a plethora of studies on the toxicity of vanadium exposure. However, the cognitive consequences of vanadium exposure have not been well studied. As reviewed, there is evidence indicating that workers who inhale or ingest moderate to large amounts of vanadium demonstrate mild to severe negative physiological and cognitive symptoms (Barceloux, 1999; Barth et al., 2002; Diamond et al., 1963; Fortoul et al., 2014; Li et al., 2013; Williams, 1952; Zhang et al., 2009).

Similarly, research using rodents has also investigated the physiological and cognitive consequences of vanadium exposure using many different dosages, and routes of administration such as injection, inhalation, and by gavage. Consistent with the human literature, rodent studies have found a range of negative physiological and cognitive effects (Avila-Costa et al., 2005; Gomez et al., 1991; Haider et al., 1998; Igado et al., 2012; Raabe & Al-Bavati, 1997; Radike et al., 2002; Sanchez et al., 1998; Soazo and Garcia, 2007; Torre et al., 1999).

In regards to the above mentioned studies, the majority utilized large doses of vanadium. Although industrial workers may inhale large amounts of vanadium, the majority of the general population in all probability will only ingest small amounts over time from their diet or groundwater (Barceloux, 1999). To date, no study (either human or rodent) has examined the neurocognitive effects (if any) of chronic mild exposure to vanadium. Hence, the purpose of the current study was to investigate whether chronic ingestion of a low dose of vanadium (.05 mg/1000mg of food mash) would affect visual and spatial memory in young male rats and to better understand chronic mild exposure to vanadium (such as found in well water and food).

CHAPTER III

METHOD

Animals

This study was carried out in accordance with the National Institutes of Health guidelines for care and use of animals in research and the protocol was approved by the Animal Care and Use Committee at West Texas A&M University. Fifteen, male Sprague-Dawley rats (one month of age) obtained from Charles River were individually housed and maintained in a temperature-and light-controlled environment with a 12 h light and 12 h dark cycle. Rats were given food and water ad libitum. Rats were administered either regular food mash (N=7) or food mash containing 0.05 mg of vanadium powder/1000mg of food mash (N=8). Food-mash was prepared one hour prior to the addition of the vanadium so that the vanadium powder could be uniformly distributed in the mixture. Vanadium treated food mash was prepared fresh daily and all rats were provided with a similar amount of food mash. Rats were weighed once a week to monitor any potential changes in weight.

Behavioral Testing

Four weeks after vanadium exposure, rats underwent behavioral testing.

Open-Field

The Open-Field task is a standard model for measuring anxiety and locomotor activity in rodents (Prut & Belzung, 2003). When rats exhibit significantly lower activity in the open field it may be an indicator of motor disturbance, and/or sickness behavior (Sah, Tirkey, Kuhad & Chopra, 2011; Walsh & Cummins, 1976). Open field testing was conducted in a square black box (57.6 cm x 57.6 cm, 38 cm high). The floor was equally divided into nine squares. Rats were placed in the open field for six minutes. Measured behaviors included locomotor activity, measured by the number of grid crosses (crossing all four paws across one of the grid lines); rearing, an assessment of exploration defined as lifting of the upper body and forepaws off the ground; and amount of time (s) spent in the center square, and anxiety measure, defined as having all four paws in the square.

Object Recognition

One day after Open-Field testing, rats were tested on the Object Recognition task. The Object Recognition task is a measure of visual memory. Specifically, this task evaluates the ability of rodents to recognize a previously presented stimulus (Jiwa, Garrard, & Hainsworth, 2010). This test involves a training and test session. During the training session, rats were placed in the open field with two identical objects for 3 minutes. The objects were two water bottles (filled so they would not be easily knocked over). The amount of time spent exploring the objects (in seconds) was recorded. Exploration included behaviors such as touching, climbing, and sniffing at the objects. Following a retention interval of one hour, rats were placed in the open field for 3 minutes (testing session) with one of the familiar objects and a novel object (one of the filled water bottles, and a glass vase, similar in size to the water bottles). Time spent

exploring both objects was recorded. A preference score was calculated as the time spent exploring the novel object divided by the total time spent exploring both objects multiplied by 100. Both the open field box and objects were cleaned with 70% ethanol before each trial to eliminate olfactory cues.

Morris Water Maze (MWM)

One week following open-field testing, spatial memory was examined in vanadium treated and control rats using the MWM. This hippocampal dependent task is the most widely used maze to assess spatial learning (Morris, Garrud, Rawlins & O'Keefe, 1982). A dark circular pool 182.88 cm in diameter and 76.2 cm in height was filled with water (21 ± 1° C) and a transparent 30 cm × 30 cm Plexiglas platform was submerged 2 cm below the surface of the water. Rats received 4 training trials each day for 4 consecutive days. At the beginning of each trial, a rat was started at one of four equidistant starting points on the pool's perimeter, in random order (designated north, east, south, and west). The rats were placed in the water and positioned to face the wall of the pool; it was allowed to locate the hidden platform, which was situated in the northeast quadrant during all training trials. A maximum of 60 sec was allowed for each trial. If the rat was unable to find the platform within the allotted time, it was guided to the platform and allowed to remain there for 20 sec. Time (in seconds) to find the hidden platform was recorded and averaged for each training session.

Statistical Analyses

All results were analyzed using SPSS statistical software. For all tests, differences were considered significant at $p < .05$. Open field data was analyzed using a multivariate Analysis of Variance (MANOVA). The independent variable was the group any given

rat was placed in (control, or vanadium exposed). The dependent variables were gridcrosses, rearing, and time spent in the center square. Object recognition (preference scores) were analyzed using a one-way ANOVA. The independent variable was group (control or vanadium), and the dependent variable was the preference score. MWM data was analyzed using a one-way repeated measures ANOVA with group as the between subjects factor and days as the within subjects factor. The independent variables were group (control or vanadium), and day of training; the dependent variable was the time it took the rat to take to find the escape platform. Furthermore, tests of power were conducted on this study to support that there was power.

CHAPTER IV

RESULTS

Open-Field

To test whether vanadium treatment resulted in global behavioral deficits, measurements included locomotor activity, exploration, and anxiety in the open field. As illustrated in Figure. 1A, both vanadium treated and control rats showed similar locomotor activity [$F(1, 13) = 0.01$, $p = .94$]. In addition, neither exploration (rears) [$F(1,13) = .21$, $p = .65$] nor anxiety-like behavior (time spent in the center) [$F(1,13) = .18$, $p = .68$] was significantly different between the treatment and control group (Figure 1B and Figure 1C).

Object Recognition

To examine whether vanadium would affect visual memory, the object recognition task was conducted. One rat was excluded from this test, due to immobility in the box. As illustrated in Figure 2, the visual working memory performance of vanadium treated rats did not differ from control rats [$F(1,12) = 2.08$, $p = .18$].

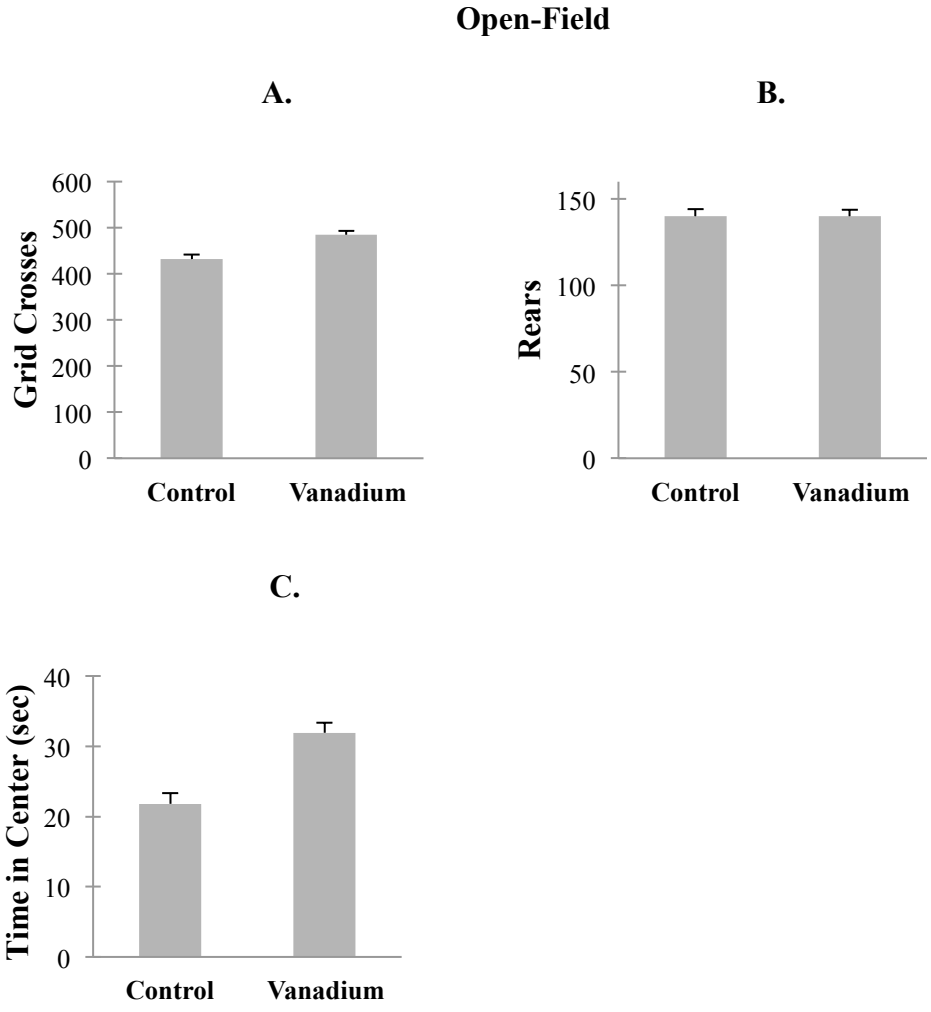

Figure 1. Vanadium exposure did not alter behavior in the open field task. (A) Locomotor activity was measured as the number of grid crosses (B) Exploration was measured as the number of rears (C) Anxiety-like behavior was measured by the amount of time (in seconds) spent in the center of the open field. Data are expressed as $X \pm$ S.E.M.

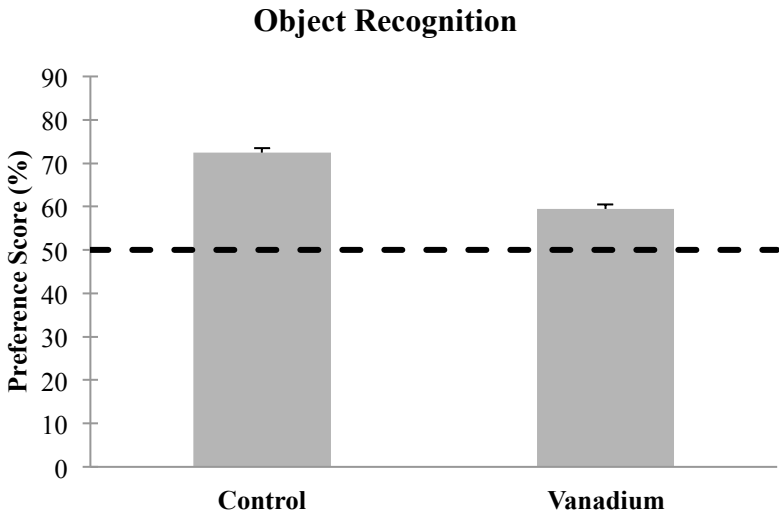

Figure 2. Vanadium did not affect visual memory performance. Data are reported as preference scores (novel object exploration/total object exploration, %, $X \pm$ S.E.M) for 3-min trials. There was a retetntion interval of 1 hour between training and test sessions. Black dottted line at 50% represents equal exploration of both objects (chance performance).

Morris Water Maze

Spatial learning was assessed using the MWM task. Statistical analyses first revealed that the difference in escape latency between days of training was significant (Figure 3) [$F(3,39) = 37.14$, $p < .01$]. Moreover, as illustrated in Figure 3, a significant interaction between day and group for escape latencies was found [$F(3,39) = 4.49$, $p < .01$]. No significant main effect of group was found [$F(1, 13) = 2.00$, $p = .18$]. To determine where significance was found on days of training, a post hoc one-way ANOVA was conducted and revealed that compared to controls, vanadium treated rats exhibited shorter escape latencies on Day 2 [$F(1,14) = 4.84$, $p < .05$].

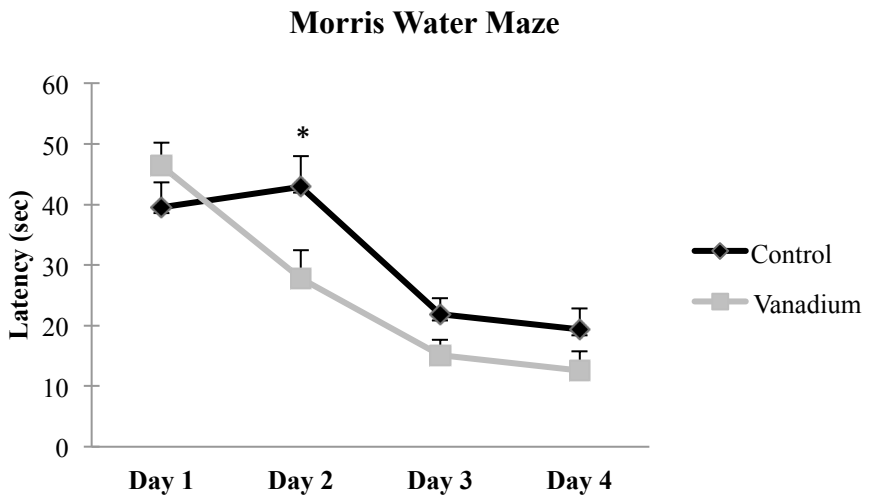

Figure 3. Spatial memory was improved on Day 2 of MWM testing following vanadium exposure. Data represent Mean escape latency times exhibited by all groups. Error bars represent $X\pm$ S.E.M. * denotes $p<.05$.

day 2.

CHAPTER V

DISCUSSION

Vanadium does not affect locomotor activity, exploration, or anxiety-like behavior

In the current study vanadium treated rats did not differ significantly from control rats on exploratory, locomotor, or anxiety behavior in the open field task. Other studies have had different results however, on this task. For example, Sanchez, Colomina and Domingo (1998), found in the first five minutes of the open field task control rats were more active than rats receiving 8.2 mg/kg/day or 16.4 mg/kg/day metavanadate (where there was activity decline) (Sanchez et al., 1998). Additionally, studies by Soazo and Garcia (2007) found neonatal rats exposed to 3 mg body weight/day sodium metavanadate from their mother's milk had significantly lower levels of locomotor activity (number of grid crosses) in the open field task, compared to controls. The studies by Sanchez et al., (1998), and Soazo and Garcia (2007) suggest locomotor activity is reduced following exposure to vanadium, but the current study suggests locomotor activity was not affected in the open field task. The current study has important implications on vanadium research. Although previous studies have found negative side effects, the differences could be explained by the dose and type of vanadium used. In the current study, vanadium powder was administered, but in previous studies metavanadate was used. Furthermore, the current research used a much lower dose of vanadium, which is clinically relevant to human daily exposure to vanadium. The higher doses and metavanadate in other studies may have led to sickness behavior and/or motor disturbances, and thus a reduction in the open field task (Sah, Tirkey, Kuhad & Chopra,

2011; Walsh & Cummins, 1976). However, the results from the current study suggest at this lower dosage, vanadium did not cause any sickness behavior.

Furthermore, since there was an absence of sickness behavior in rats, this opens possibilities of using vanadium in treatment for diseases such as diabetes. Soveid et al. (2013), found diabetic patients who were administered vanadium treatment equal to 1.5 mg vanadium powder to help control poor blood glucose for 30 months. The effect of vanadium treatment led to 30% reduction of insulin need and control of blood glucose. At lower doses, vanadium treatment for diabetes may be beneficial. Since toxicity was not found in the current study it may be a safe option for treatment of diabetes. One important thing to note is that in the study by Soveid et al. (2013), patients were administered three times the amount used in the current study. Although this is a larger amount than what was used in the current study, it is still a much lower dose than a lot of previous research on vanadium that produced a variety of negative effects.

Vanadium does not affect visual memory

The object recognition task is used as a model of memory on rats, in assessing visual memory (Jiwa et al., 2010). The current study did not yield significant findings between groups on the object recognition task. This study was the first (to date) to explore effects of vanadium on the object recognition task. One interesting finding was that while there were no significant differences in this task, control rats performed at 70% whereas the vanadium exposed group performed only slightly above chance performance. Since the current study had a small sample size, it would be beneficial to see if a larger sample size achieves significance. Although there are no other studies on this task following vanadium exposure, it is nonetheless important as a model of memory to better

understand the neurocognitive effects of vanadium. It is also beneficial to recognize the brain regions associated with this task: the perirhinal cortex, and hippocampus, which have been explored in humans, monkeys, and rats (Platano, Fattoretti, Balietti, Bertoni-Freddari & Aicardi, 2008; Winters, Saksida & Bussey, 2008). Some studies using higher doses of vanadium found hippocampal damage, but since the current study used a significantly lower dose this may be a reason there were no differences in this task (Avila-Costa et al., 2006; Azami et al., 2011; Igado et al., 2012).

Vanadium positively impacted spatial memory

On day 1 of the MWM, vanadium-exposed and control rats performed similarly. On day 2 however, vanadium rats exhibited significantly better spatial memory compared to controls. On day 2, control rats took longer to find the platform and throughout the rest of the task (days 3 and 4), there was a trend for the vanadium treated rats to show a shorter escape latency compared to the controls. Perhaps with a larger sample size, this could reach significance on all days of the MWM task.

The findings of the current study on the MWM task are important because it relates to vanadium exposure in humans, at a low level in food and water. The current study was done to have a better understanding of the effects of chronic low dose exposure to vanadium. Furthermore, it again highlights the importance of using vanadium for treatment of diabetes. At a lower dosage, no toxic effects were found, and some improvement was observed. In contrast, other studies found impairment on this task.

Consistent with current study, Avila-Costa et al., (2006), found no clinical or weight changes in research on rats, but behavioral changes were observed. For example, on the MWM, rats were trained on the task and had preference for the quadrant

containing the platform. However, following inhalation of vanadium, rats did not show a preference for the platform-containing quadrant. These results suggest an effect of decreased spatial memory after inhaling vanadium pentoxide. The current study did not have effect like this on spatial memory. Although on the first day of training on the MWM vanadium exposed rats did not indicate a preference for the quadrant containing the platform, on the second day preference for the quadrant containing the platform greatly increased, and learning (on average) occurred much quicker than the control group, which suggests an improvement on spatial memory. Indeed, for the remainder of the trials on the MWM, vanadium rats continued to find the platform faster than the control rats, suggesting no impairment on spatial memory, and a trend of possible improvement on memory tasks.

Azami and colleagues (2011) tested mice on the MWM following exposure to oral administration of vanadium, and found that vanadium treated mice displayed significantly poorer results on the task. This study is unlike the current one, suggesting vanadium is associated with spatial memory impairment, but this could be due to using metavanadate and a much larger dose, again highlighting the importance of using the clinically relevant lower dose exposure level (Azami et al., 2011).

A structure that is critical to the MWM task is the hippocampus. In rodent studies, hippocampal damage was found where there were greater dosages of vanadium (Igado et al., 2012). The findings from previous research suggest that vanadium affects the hippocampus, and thus led to poorer results on the MWM task. It is possible that the current study did not yield significant findings on the MWM task due to the lower dose used, but it could also suggest that hippocampal damage did not occur at a low dose. The

low dose used in the current study relates to average human exposure to vanadium, and a realistic finding for the risk the general population has for hippocampal damage.

Although human studies of vanadium administration found decreased visuospatial skills using the Block-Design test, as well as coordination, memory, and reaction time decline, the exposure levels used in the human studies was far greater and over a longer duration of time than in the current study (Barth et al., 2002; Li et al., 2013).

Conclusion

The current study is important to vanadium research because it is the first to investigate the effects of low dose chronic ingestion of vanadium in rats, using three different behavioral tasks. It is clinically relevant to human research on vanadium, because humans are likely to ingest small variable amounts of vanadium over time through the diet. At the dosage used (0.05 mg/mL), no negative health effects were found, no impairment in memory was suggested, and evidence of better memory performance on the MWM was observed. The current findings suggest, as evidence of results from the MWM, that memory in vanadium-exposed rats was better than controls on day two. Furthermore, throughout the rest of the MWM task, on days 2 through 4, vanadium rats consistently took less time finding the platform than control rats, which implies that vanadium may indeed improve memory.

Vanadium has been offered as a potential treatment for diabetes. Guo and colleagues (2011), investigated the effects of vanadium co-treatment with *Cordyceps sinensis* (an absorbent fungus used medicinally in China) to rats following i.p. injections of streptozotocin, which induced diabetes. The streptozotocin reduced insulin; however, the rats receiving vanadium had lower blood glucose levels and a significant increase in

insulin, suggesting positive treatment possibilities (Guo et al., 2011). Since research on vanadium use in diabetes treatment has posed positive results, and the current study has found that low dose vanadium exposure did not lead to sickness, motor impairment, or memory impairments, the benefits may outweigh the risk in using vanadium as a treatment at this dosage level. Likewise, when vanadium has been associated with sickness behavior, upper respiratory problems, memory impairment, or other negative effects, it occurred in studies using much higher dosages than in the ones that found little to no difficulties.

The findings of the current study are similar to findings by Edwards, Hall and O'Bryant (2012). In this diabetes study, participants with and without diabetes mellitus were analyzed and compared on tests of neuropsychological functioning. Results indicated that diabetic participants exposed to higher groundwater vanadium levels had better scores on immediate memory and executive functioning. Participants who were not diabetic and were exposed to higher amounts of groundwater vanadium were significantly related to higher performance on immediate memory, executive functioning, and language (Edwards, Hall & O'Bryant, 2012). This is similar to the current study in that vanadium levels were related to better performance on memory tasks. It would be beneficial to keep looking into research concerning this effects of vanadium on memory performance, cognitive function, and language, because the current study and the Edwards study both found possible improvement in these tasks.

Although memory may have improved, there are important aspects to consider on tasks of learning and memory following exposure to vanadium. Firstly, the type of vanadium that is administered may induce different responses, such as vanadium powder

(used in the current study), sodium metavanadate, orthovanadate, vanadium pentoxide, vanadyl sulfate, or other forms of vanadium. Gomez et al., (1991) found that vanadyl sulfate induced different excretion than soudium metavanadate, so type of vanadium used should be considered when researching with this substance. Also important in research on vanadium is the route of exposure. As with drugs such as cannabis, differences in effects may be seen following different routes of exposure (Guy & Flint, 2003). The route of vanadium exposure combined with the type of vanadium used and dosage, can lead to a variety of symptoms and behavioral responses, as seen in the literature on vanadium (Chandra, Ghosh, Chatterjee & Sarkar, 2006; Gomez, Domingo, Llobet & Corbella, 1991; Guo, Li, Wang, Liu & Zhang, 2011; Haider et al., 1998; Sanchez, Colomina & Domingo, 1998; Soazo & Garcia, 2007; Williams, 1952).

The current study has certain limitations that are important to consider. First, vanadium was administered via mash food (500 mL food and 500 mL water); it is uncertain as to how much vanadium any given rat actually ingested, because levels of vanadium were not ascertained. Since content analysis of vanadium in excretion or blood was not conducted, vanadium consumption cannot be determined. However, it is important to note that this study is an example of a real world situation, where amounts of metals and other substances present in the diet are not generally measured. Also, as Radike et al., (2002) found; differences can be seen in the administration method of vanadium via water in comparison to administration of vanadium via food, where food has a lower absorption rate. Indeed, Diamond et al., (1963) found that vanadium administered by food has an unpredictable absorption rate. It can be assumed then, that other administration methods, such as intraperitoneal (i.p) injections or inhalation and

even drinking water containing vanadium may induce stronger reactions than ingestion by food.

One other limitation to the current study is that it was done with a small sample size, and was carried out with only a single cycle, due to time concerns. Using a larger sample size with multiple cycles would help expand findings, and may lead to significant findings.

More research concerning behavioral and neurotoxic results following vanadium exposure needs to be executed to better understand the effects (positive and negative) it may have on health. This research study should be expanded by examination of rat brains, which were extracted and preserved for future research (perhaps evaluation of vanadium levels in the brain, as well as determining if there are any structural differences between groups). The study by Soazo and Garcia (2007) on rat brains following administration of vanadium to neonatal rats via mothers milk, found that neonatal rats had visible decreases in myelin in the brain, which would be interesting to examine from rat brains in the current study.

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