The Effects of Transcranial Direct Current Stimulation and an enriched Environment on Working Memory in the Rat Model

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An enriched environment facilitates learning and it protects neural fields against the progression of dementia. Transcranial direct current stimulation (tDCS), which passes a weak current through the brain, mimics behavioral improvements and neuroplasticity proteins associated with an enriched environment. This experiment uses a delayed spatial alternation task to measure differences in spatial reference memory and working memory between environmentally enriched rats and rats intended to receive transcranial direct current stimulation. The head tDCS electrodes were not stabilized onto the rats' skulls, and as a result, only the merits and faults of this surgical procedure are discussed. Nonetheless, enriched rats need less time to reach both phases of behavioral shaping and they require fewer direction choice errors throughout learning the spatial alternation task. Though there is no difference in working memory performance between the groups with extended delays, these results suggest that an enriched environment improves the acquisition of spatial information and may reduce the group's variability for intelligence.

INTRODUCTION

Every animal's success relies on its physical traits and its neural adaptability. Neuroplasticity, the essential process of altering connection strengths between neurons as a means to create new cognitions and behaviors for ongoing environments, is driven by genetics and activities within the environment (Laviola, Hannan, Macri, Solinas, & Jaber, 2008; Lillard & Erisir, 2011; Van Praag, Kempermann, & Gage, 2000; Volkers & Scherder, 2011). Consistent and challenging interactions with the environment produce neuroplasticity proteins that encourage interconnectivity between neural fields, which are volumetric neural templates that interact to process, manipulate, and sustain information for a cognition or behavior (Draganski et al., 2006; Yuste, 2011). With more potential cognitive reserves from heightened interconnectivity, an enriched environment improves learning abilities and impedes the development of dementia-related symptoms (Arnaiz et al., 2004). Considering that some individuals may not have access to an enriched environment, the recent method of transcranial direct current stimulation (tDCS) may act as a potential substitute for an enriched environment, since tDCS improves similar learning performance and produces neuroplasticity proteins that promote regional interconnectivity. Numerous researchers have investigated the forms of electrical and enriched stimulation, yet this current experiment designs a novel tDCS electrode for rats and provides new results for a figure-eight maze that has never before been used to test the effects of an enriched environment or transcranial direct current stimulation. This experiment addresses how living in a diverse, interactive environment or receiving transcranial direct current stimulation to the frontal lobes affects the animal's ability to learn a simple alternation task and sustain spatial information for an upcoming decision, which is a hypothetical construct defined as working memory (Khan & Muly, 2011).

Spatial working memory allows an animal to sustain information from the environment when it is no longer present. In humans, finding a house in a city requires spatial working memory, since the person must utilize an internal cognitive map of the city in order to navigate to a particular destination. As global positioning systems (GPS) are becoming popular, Ishikawa et al. (2008) discovered that individuals using a GPS instead of a personal guide or maps actually have reduced navigational abilities and need more time to reach a destination. If considering GPS a passive interaction between the person and the environment, more intentional interactions with the environment can train spatial working memory by making the person more adept to recognizing and locating objects for future working memory manipulations. In particular, rats need spatial memory to locate and retrieve hidden objects, a notion that suggests an effective working memory identifies and prioritizes spatial cues when searching for a reward. Rodents may require spatial working memory to survive by finding food, while humans' survival is less dependent on spatial working memory abilities; nonetheless, impairments in spatial working memory leads to a higher risk of developing Schizophrenia and other forms of psychosis. In addition, reduced abilities in nonverbal tasks like arithmetic, geometry, and science correlate with deficits in spatial working memory. In essence, addressing how an enriched environment can improve spatial working memory may reduce an individual's potential to lose contact with reality and it may help an individual manipulate scientific or geometrical themes within his or her own mind.

Simpson and Kelly (2011) created a new protocol for enriched environment experimentation to include specific parameters for both nonsocial and

social activities. To begin with, nonsocial components of the apparatus, like the cage size, toys present, foraging material, and the number of weeks in these conditions, determine the extent to which the animal interacts with the environment. Simpson and Kelly (2011) suggest that more significant improvements in learning, habituation, and attention occur when the cage measures 252,000 cm^3, the toys (running wheel, ropes, bells, blocks, sticks) are switched every three days to ensure a novel environment, and the rats begin enrichment younger than four weeks of age for a duration of at least four weeks. Even the rat strain can affect enriched environment results, in that pigmented rats, as compared to albino rats, habituate faster to learning and memory tasks and are less likely to produce measures of anxiety such as urination and defecation that may impede task performance (Ennaceur, Michalikova, & Ahmed, 2004). In addition to these nonsocial parameters, four rats in each cage provide enough social activity to satisfy the most supported and recent protocol for enriched environment experimentation.

Even before the development of this enriched environment protocol, however, Donald Hebb was the first to document that free-roaming rodents had superior learning abilities when compared to rats housed in standard cages (Hebb, 1947). These measurable improvements in behavior range from simple tasks, like reference memory, to working memory, which uses delay periods to stress the rat's ability to recall previously visited positions (Del Arco, Blas, Garrido, & Mora, 2008; Simpson & Kelly, 2011). As compared to controls, enriched rats performed an alternation task, which requires the rat to recall previous positions and alternate its direction choice over short delay periods, with greater accuracy, (Arco, Segovia, Garrido, Blas, & Mora, 2007). Rats have an innate tendency to alternate directions when faced with a familiar crossroad—an effort to explore potentially rewarding areas—yet enriched rats continually alternate over numerous trials with fewer errors as compared to non-enriched rats. Enriched rats spent less time with newly presented toys and explored more than non-enriched animals, a response that suggests that enriched rats have superior memory-processing abilities and an internal attention force that is satisfied when inputs to their central nervous systems vary (Simpson & Kelly, 2011; Zimmermann, Stauffacher, Langhans, & Wurbell, 2001). In addition to the underlying notion that enriched animals have more modifiable and responsive neural fields, when the context of a working memory task is changed, enriched rats, unlike rats in standard housing, create strategies faster to track and obtain the reward (Leggio et al., 2005). As expected, rats housed in an impoverished environment have reduced spatial working memory and spatial reference memory abilities as compared to controls (Diniz et al., 2010). In contrast, however, Segovia, Arco, Blas, Garrido, and Mora (2008) used a water T-maze and found no difference in working memory performance or the days needed to reach criteria after months of an enriched environment. Though they rearranged the toys every five to six days, they suggest that working memory performance declines with age and an enriched environment does not improve working memory performance. In some cases, an enriched environment improves the rate and accuracy of learning and performing tasks, albeit in some spectral relationship within an enriched environment's capacity to inhibit the symptoms of dementia and traumatic brain injuries.

An enriched environment provides ameliorative and protective effects against the progression of Alzheimer's disease and traumatic brain injuries (Will, Galani, Kelche, & Rosenzweig, 2004). Alzheimer's disease is the most common form of dementia in humans, and though genetic factors contribute to this disease's progression, both amplified cognitive and physical activity protected and improved working memory, reference memory, and recognition memory in rodent Alzheimer models (see review, Laviola, Hannan, Macri, Solinas, & Jaber, 2008). More importantly, the amount of cognitive and physical activity inversely correlates to the likelihood of developing early and severe dementia-related symptoms (Scarmeas et al., 2011). Just as Alzheimer's disease progresses to destroy regional dendrites and neurons in the hippocampus and frontal lobes, traumatic brain injuries reduce interconnectivity in a similar fashion. As expected, an enriched environment improved working memory and reference memory performance for rats receiving injuries that mimic human brain injuries from war, accidents, and stroke (Puurunen & Sivenius, 2002). Considering the fact that human enriched environment studies are difficult to operationally define, clinicians suggest that humans who regularly learn, exercise, and read will rehabilitate faster and will be less likely to exhibit the severe symptoms following the onset of dementia or traumatic brain injuries. In essence, because an enriched environment provides varied incoming information to the neural fields, the global neural system must adapt to a wide variety of information processing. With this incoming stimulation, the cortex physically expands to form new connections with other neural fields. As a result, the rate and accuracy of learning simple and complex tasks may reflect the interconnectivity or density of specific and global neural fields.

The density of the cortex can measure the degree of inter-connectivity between local and global neural fields (Yuste, 2011). Gray matter, or the cerebral cortex, consists of multiple types of cells that help adjacent neurons communicate and survive within a neural field. Enriched animals have heavier and thicker cerebral cortices as compared to non-enriched animals (Diamond, 2001; Draganski et al., 2006). Histological human autopsies provide a correlation between the density of neurons within the cerebral cortex and the hippocampal CA1 region to the person's level of education (Diamond, 2001; Jacobs, Schall, & Scheibel, 1993). Specifically, the expansion of vascular couplings that supply oxygen, the incorporation of newly generated neurons, and the proliferation of dendritic spines account for the thicker and heavier cortices. Lying on dendrites, dendritic spines are mushroom shaped protrusions that fluctuate in volume and length to provide novel and potentially beneficial connections to process information (Bourne & Harris, 2007; Kasai, Fukuda, Watanabe, Hayashi-Takagi, & Noguchi, 2010). Kolb et al. (1999) recognized that an enriched environment physically extends the length, volume, and quantity of dendritic spines in the frontal lobes and hippocampus. This trend towards heightened interconnectivity between neurons results from the expression of neuroplasticity proteins like brain derived neurotropic factor, neural growth factor, and hypothalamic-pituitary-adrenal axis protein in corresponding neural fields that accompany improvements in learning and spatial memory performance. These proteins, which expand neural fields by creating new connectivity pathways, are less abundant in non-enriched animals that perform poorly on memory and learning tasks (Segovia, Arco, & Mora, 2009). The degree of gray matter density and its corresponding interconnectivity contributes to how an enriched environment improves and protects cognitive and behavioral performance.

The STAC model and Yuste (2011) explain these correlations between the density of specific neural fields to cognitive and behavioral abilities. According to the STAC model and Yuste (2011), local and global neural fields with heightened interconnectivity and gray matter density are more resilient, responsive, and effective when processing information, which means the system can repair itself, find alternative neural pathways for a goal, and integrate and sustain more complex information. The frontal lobes and the hippocampus are especially sensitive to this process, as these regions rely on highly modifiable connectivity throughout life. With fMRI techniques, Park and Reuter-Lorenz (2009) claim a scaffolding theory of age and cognitions (STAC) that recruits frontal lobe neural fields to process information when rudimentary neural fields, like the parietal and temporal lobes, decline in functionality during the process of aging. The frontal lobes have this compensation potential because of their naturally dense and highly modifiable neural fields throughout life. For individuals suffering from dementia, the slow progression of dementia-related deficits in memory, thinking, and behavior may depend on how many neural fields are able to process information and behaviors at any given moment, since the disease's pathology disconnects local and global neural fields by eliminating dendrites, dendritic spines, and neurons with time. In addition, damage to a particular neural field, like the hippocampus in Alzheimer's disease, inevitably limits the potential for all neural fields to communicate and interact to produce executive functions like verbal and spatial working memory and creating solutions to novel problems (Martinez & Colom, 2009). In theory, the basis for inherited intelligence and this compensatory potential to protect against neural field degradation is related to the volumetric architecture of neural fields. As argued here, however, these connectivity scaffolds are not completely defined by genetics. Instead, an enriched environment provides varied information to the global neural field that improves its ability to process information. Some individuals, however, may not have access to an enriched environment. As a result, these individuals need a different method of stimulation that mimics the expression of neuroplasticity proteins that protect and improve certain behaviors and cognitions. Taken together, perhaps people without the opportunity to stimulate these beneficial effects may use transcranial direct current stimulation to improve their learning abilities and reduce their likelihood to develop dementia in the future (Simpson & Kelly, 2011; Stagg & Nitsche, 2011).

Transcranial direct current stimulation (tDCS), a method that passes a weak current through the brain, interacts with underlying cortical neural fields via the electrode's electric field (Stagg & Nitsche, 2011). This inexpensive apparatus simply consists of a constant current source and two electrodes in which either the anode or cathode is placed above a cortical region of interest, while the reference electrode is placed on the shoulder or the occipital-frontal region (Paulus, 2011). Neuro-imaging research suggests anodal stimulation increases underlying cortical activity, modulates GABA and glutamergic synapses that induce long-term potentiation, and facilitates the production of brain derived neurotropic factor and cerebral vesicles that supply oxygen (Fertonani, Rosini, Cotelli, Rossini, & Miniussi, 2010; Zaehle, Sandmann, Thorne, Jancke, & Herrmann, 2011). Though an enriched environment and anodal tDCS

both facilitate the production of neuroplasticity factors that expand gray matter, they differ in terms of how long these heightened expressions exist: anodal stimulation induces short-term changes to the neuron's membrane potential while an enriched environment facilitates long-term changes in receptor activity (Stagg & Nitsche, 2011; Venkatakrishnan & Sandrini, 2012). This trend suggests that both methods induce neuroplasticity by different mechanisms. In an opposite effect to anodal stimulation, cathodal stimulation reduces underlying cortical hyperactivity (a beneficial effect for status epilepsy) and it diminishes the expression of neuroplasticity factors that promote neural field expansion (Kamida et al., 2011). Within the tDCS literature, differences in behavioral and cognitive performance result from the size of the electrode, the polarity and amount of current used, the duration of stimulation, and the location of the electrodes, because these stimulation parameters affect the orientation and magnitude of the electric field (Stagg & Nitsche, 2011). Nonetheless, artificially induced activity to a neural field, as with anodal stimulation, increases the degree of neural field interconnectivity and improves behavioral and cognitive performance.

Human transcranial direct current stimulation improves multiple types of performance. An anodal current density (current (amperes)/area of electrode (meters^2)) of at least .53 A/m2 to the motor, the occipital, and the parietal lobes, improved motor, visual, somatosensory, and simple learning performance for up to forty minutes, respectively (see review, Brunoni, Fregni, & Rosana, 2011). More specifically, anodal stimulation to the frontal lobes improves performance on a working memory n-back card tasks, which require the participant to remember previously presented symbols and determine when these symbols arrive some n steps in the future (Fregni et al., 2006; Zaehle, Sandmann, Thorne, Jancke, & Herrmann, 2011). Andrews et al. (2010) suggest the time and errors needed to complete a task are significantly less when the stimulation is administered while the task is learned. Considering that tDCS research is young and is limited by human ethical considerations, animal tDCS models allow for increased stimulation protocols to elicit neuroplasticity and behavioral effects.

Ulz, Dimova, Oppenlander, & Kerkhoff (2010) suggest that heightened electric field magnitudes, like those used in animal tDCS models, may activate novel neuroplasticity mechanisms that provide new stimulation parameters to improve performance. Currently, only ten animal tDCS experiments have measured the neuroplasticity effects of anodal and cathodal stimulation with current densities ranging from 1.7 A/m2 to 57.1 Am2, which fall well below the lesion threshold current density of 147.3 A/m2 as established by Liebetanz et al. (2006). Practically all of these tDCS studies addressed changes in cortical activity and gray matter density, as discussed earlier, yet the next frontier of animal tDCS strives to find improvements for executive functions like working memory. Dockery et al. (2011) used the Liebetanz et al., (2006) rat electrode setup to test working memory in a delayed place avoidance task—requires the rat to recall past spatial locations to avoid a punishment—yet they used cathodal stimulation and only documented significant improvements in task performance when the rats were tested three weeks after stimulation ended. They suggest these latent results occur because these spatial memories migrate from the hippocampus to the neocortex and enhance activity patterns after twenty-one days. Though this animal tDCS study does not produce timely improvements on learning or working memory performance, some human studies have shown lasting effects on learning and working memory performance from both anodal or cathodal stimulation by using anodal stimulation during the acquisition phase and cathodal stimulation during the retrieval phase (Stagg & Nitsche, 2011; Brunoni, Fregni & Pagano, 2012). Transcranial direct current stimulation is in the beginning stage of its research, and the numerous and unpredictable animal tDCS challenges are worthwhile because animal models give the experimenter more control to manipulate stimulation and learning procedures. With time, these animal tDCS models will offer correlations between the expression of local and global neuroplasticity mechanisms and stimulation parameters to help explain the neurological manifestation of intelligence.

Transcranial direct current stimulation, much like an enriched environment, facilitates heightened interconnectivity in local and global fields. Polania et al. (2011) demonstrated that localized tDCS to the left somatosensory region increases its connectivity to the left motor, premotor and parietal regions. More importantly, no region becomes less connected from stimulation, which suggests that anodal tDCS does not impede interconnectivity. In human stroke tDCS experiments, anodal stimulation to the damaged region with cathodal to the contra-lateral position improved the rate of task learning, perhaps resulting from reduced inter-hemispheric inhibition and increased neuroplasticity expressions in the damaged area. When applied to the left frontal lobe, anodal tDCS increases the synchronization of both frontal lobes, which may reduce signal to noise ratios and allow for more effective functional connectivity networks (Bastani &

Jaberzadeh, 2012). Recent neuroscience research assumes that intelligences like language, coordinated movements, and working memory do not rely on one specific neural field; instead, these regions' existing neural architecture and their potential to adapt to the environment manifest into a spectrum of intelligence.

Finding methods to improve working memory performance and reference memory, such as with an enriched environment and transcranial direct current stimulation, provide potential improvements in one's quality of life. Working memory is a hypothetical temporary storage device that sustains expired sensory information and manipulates the information to acquire a goal (Baddeley, 1992). Working memory is highly correlated to other executive cognitions like consciousness and our ability to create solutions to novel problems (Baddeley, 1992; Khan & Mully, 2001). As mentioned earlier, intelligence performance lies on a spectrum. If we consider this spectrum to represent variability in intelligence for the general population, an enriched environment has the potential to reduce this variability by sliding low performing individuals to the middle of the spectrum. Though an enriched environment may not greatly improve the performance of a naturally gifted person, reducing the variability of the entire group increases the likelihood that more individuals will learn from their past failures, predict future rewards and consequences, and improve their belief that they can accomplish a desired result. This self-efficacy positively correlates to how individuals value their own worth (Lecic-Tosevski, Vukovic, Stephanovic, 2011). In essence, improving working memory functions decreases the likelihood that a person will become frustrated with challenges and may potentially improve one's general happiness in this challenging and demanding world.

In the rat model, working memory is measured by a variety of tasks. The most popular tasks are the Morris water maze, the radial arm maze, and the water Y or T maze, all of which require the rat to remember previously visited platforms and runways to obtain a reward. Though less research uses this experiment's delayed spatial alternation task, commonly known as the figure-eight maze, it nonetheless measures multiple characteristics about the rate and accuracy of learning simple reference memory tasks, like remembering where an item is located, and spatial working memory tasks, like recalling previous direction choices after short or extended delay periods (Pedigo, Song, Jung, & Kim, 2008). A figure-eight maze receives its name because five connecting runways form the number eight: there are two gates in the center and the top runways, and these gates open and close to either force a specific direction choice or enclose the rat for a delay period before it freely chooses a direction (Figure 1). The first phase of behavioral shaping forces the rat into the left or right runway and it must then return to the center holding area to obtain a water reward; on the next learning phase, the rat is rewarded for choosing the opposite or alternating direction in reference to the previous trial's direction. In this case, the number of trials, errors, and time needed to reach phase criteria are measured. When the rat adequately learns these behavioral shaping phases, its working memory is tested by increasing the center area delay period to twenty, thirty, or forty seconds before the rat must recall the previous trial's direction choice. Within the existing research, this specific figure-eight maze has never specifically measured the effects of either an enriched environment or transcranial direct current stimulation.

The recent methods of tDCS and the figure-eight maze require a novel surgical procedure to insert the active electrode above the frontal lobes of mobile rats. As there have been few animal tDCS studies, Liebetanz et al. (2006) offer the most popular surgical procedure, yet their literature does not discuss in detail the surgical procedure. As developed by Liebetanz et al. (2006), a hollow plastic capsule with a 3.5 mm^2 circle is filled with a saline solution to conduct electricity. This electrode is glued above the cortical region of interest and the reference electrode is glued into a rat jacket to make a complete circuit through the rat's head and abdomen. In relation to this current experiment, this surgical procedure has limitations. First, it is difficult to properly situate this electrode on an uneven rat skull without the glue drifting into the reservoir hole. Second, when filling this electrode with the saline solution, it is nearly impossible for the solution to reach the base of the skull and allow for a uniform electric field. Third, since rats groom themselves and wrestle with each other, the electrode setup from Liebetanz et al. (2006) does not entirely stabilize the electrode for extended periods like the one this current experiment requires. In an effort to improve these limitations, this current experimenter's surgical procedure involved screwing two separate screws (3.2 mm^2) above the cortical region of interest (to serve as the active electrode) and beside bregma (to stabilize the entire electrode setup when glued together). The rat jacket that holds the reference electrode was used from Liebetanz et al. (2006), yet the actual surgery completed in this experiment is completely novel within the tDCS literature. It is expected that a current density of 45 A/m^2—which is less than Liebetanz et al.'s (2006) surgical procedure since there is less skull to shunt the current from inducing brain lesions—will interact on the frontal lobes and improve working memory.

This experiment aims to relate the effects of an enriched environment and transcranial direct current stimulation on spatial reference memory and spatial working memory. This review has focused on the relationship between neural field density and cognitive performance. In particular, all cognitions and behaviors may potentially result from regional and local connectivity interactions. This experiment is novel in terms of the tDCS electrode surgical procedure and the delayed alternation task, since it has never been used before to test the effects of an enriched environment or transcranial direction stimulation. The hypothesis for this experiment states that enriched rats, as compared to controls, will need fewer days, fewer errors, and less time when returning to the center starting area in phase one and when making direction choices in phase two. Transcranial direct current stimulation rats will reach phase two criteria with fewer days and errors. As compared to controls, both groups of enriched and tDCS rats will produce fewer errors during the extended delays that test working memory.

MATERIALS AND METHODS

Subjects and Housing Twenty-two male Long-Evans rats (aged three weeks) were ordered from Charles River Laboratories (Virginia Beach, VA). Animals were housed in the basement of Bagby Hall in a temperature-controlled room on a 12-hour light/dark cycle with water and food *ad libitum*. Each cage and enriched environment was cleaned or replaced every four days. Hampden-Sydney College's Institutional Animal Care and Use Committee have approved all experimentation.

Rats were either housed in an enriched environment or a standard cage for four weeks before learning the figure-eight maze procedures. Ten rats were in the control group, in that they lived in standard cages with wood clippings and would not receive tDCS or live in an enriched environment. Six rats were housed in the standard cage and were to receive transcranial direct current stimulation during the second phase of learning, as discussed below. The last six rats were housed in an enriched environment throughout the entire experiment.

Enriched environment Six rats receiving the independent variable of an enriched environment were housed as a group in one of three volumetrically similar enriched cages (76 X 60 X 60cm) throughout all experimentation. Every four days, these rats were transferred to another clean, enriched environment that had new interactive toys like a running wheel, ramps, blocks, sticks, newspaper clippings, bells, boxes, as well as the general space to explore (Figure 2). Each rat was socially enriched by the spontaneous activity from the other five rats in the group. In order to encourage exploratory behaviors, food and water were administered through the cage bars in different areas twice a day.

Transcranial direct current stimulator Dr. Mark Holcomb, a visiting professor at Hampden-Sydney College, created the direct current stimulator (Figure 3) and the USB software to set the current, the duration, and ramping effects (increasing the current over thirty seconds to the desired current). This stimulator requires twenty volts to pass a current through the rat and it beeps when the circuit is broken or the current is not fully delivered. The thirteen feet of anode and cathode wires are attached to the stimulator with banana clips. The cathode or the reference electrode (10.5 cm2) was glued to a medium sized rat jacket (Braintree Scientific). Velcro straps stabilized the electrode on the rat's abdomen (Figure 4).

Skull Electrode Surgery This tDCS apparatus is slightly modified from Liebetanz et al. (2006), who glued a plastic capsule (3.5 mm^2) onto the skull and filled it with saline solution to conduct electricity. This current experiment used the same jacket for the reference electrode, yet it differed in the actual head electrode surgery (Figure 5). After anesthetizing tDCS rats with ketamine (.95 mg/kg) and xylazine (.4 mg/kg), a manual drill tool drilled one hole above the frontal lobes (3.1 mm anterior to bregma) and one hole to the right of bregma. Next, two screws with a common surface area (3.2 mm^2) were screwed into these holes. The screw above the frontal lobes acted as the electrically active screw, since the bregma screw was meant to help stabilize the active electrode onto the skull once the glue dried. A thin copper wire measuring an inch and a half wrapped around the active electrode and protruded to the posterior regions of the skull. At the other end of this short wire, a male spade clip was soldered as a connecting device with the female spade clip and its attached anode wire. Glue (Loctite) was then distributed around the two screws on a clean, dry skull. Once the glue dried, the rat's scalp was sutured together with the small wire and its male spade clip positioned in a parallel manner to the rat's spine. Afterwards, antibiotic ointment was spread on the incision and the rat was placed under a heating lamp until it became mobile again. These rats were allowed three days of rest before any phase of testing began.

tDCS Stimulation Parameters To produce a current density of 45 A/m^2, a current of 144 microamperes had planned to be administered during the short working memory delay task

(because of surgical complications, no tDCS rat was successfully stimulated). Anodal stimulation and its thirty-second ramping effect would have started when the rat began the second phase of testing. While performing the task, each rat would have been stimulated for fifteen minutes. Even if the rat completed the thirty trials in less time, it would be given water and food in the maze when waiting for the stimulation duration to pass. In addition, some tDCS rats will reach criteria in fewer days than other tDCS rats; nonetheless, each tDCS rat would receive anodal stimulation for as many days as it takes it reach phase two criteria.

Figure-Eight Maze This manual spatial alternation task or the figure-eight maze was modeled after the computer-automated version described by Pedigo, Song, Jung & Kim (2006). The Plexiglas base measuring 91 centimeters^2 was placed one meter above the floor. Plexiglas walls (350 mm) were placed around the base and were covered with black mesh to eliminate visual cues within and outside of the maze. Two Plexiglas rectangular boxes were glued onto the Plexiglas base to separate the resulting runways (12cm wide and 91 cm long) into a figure-eight shape. Three separate water wells were positioned on the midlines of the three parallel runways (left, center, right). The experimenter used a solenoid apparatus controlled by a photography exposure time box to administer the water as the rat approached the well.

Four Plexiglas gates opened or closed to allow or block the rat from entering into new runways. The two gates in the center runway are positioned 35 cm from the central reward well. When testing working memory performance in phase three, this center area will confine the rat for a specific delay period. The two gates on the top runway were positioned 33 cm in front of the corners that lead to the left or right runways. All four gates pivoted on a pin that was drilled into the Plexiglas. These gates opened and closed by a connecting handle that latched to screws drilled onto the top of the rectangular boxes. The transcranial direct current stimulation wires, which include the rat jacket and the male spade clip, draped directly above the center of the task from the ceiling. A loose 70cm spring attached to this hanging wire to reduce the wire's tension on the head electrode. In addition, copper wire was shaped into semi-circles and taped around the maze's corners to reduce wire tension and allow the rat to freely travel around the corners of the rectangular boxes.

Procedure This procedure uses three phases to test spatial reference memory and spatial working memory. The first phase requires the rat to return to the center runway. The second phase requires the rat to alternate its direction choice from the previous trial over short delays. The third phase measures spatial working memory performance with increased delays or cognitive loads. Quantifiable errors include back-edge errors (after choosing a left runway, the rat goes into the right runway instead of returning to the center runway and vice versa), direction choice errors (the rat does not choose the opposite direction runway in reference to the previous trial), and lap times (the amount of time needed to leave and return to the center runway. A direction choice is finalized when the rat's nose passes one of the corners in the right runway. Rats were deprived of water for twenty four hours before the habituation phase. All procedures were carried out with white noise and a red light, since red light is not in the visual spectrum of rats. After completing thirty trials for each session, rats were given water for twenty minutes and then returned to their respective cages.

Habituation Weeks before the first phase of shaping, tDCS rats were repeatedly dressed with the rat jacket. Before training began, all rats were habituated to the figure-eight maze for fifteen minutes over four consecutive days. During this habituation phase, rats explored the task while the experimenter randomly administered water to the three runways. After each session of habituation, the rats were given water for twenty minutes and then returned to their respective cages.

Behavioral Shaping The first phase of shaping requires the rat to return to the center runway after each trial. When the front-centered gate opens to start the trial, the left or right runway is alternately blocked by a gate, which forces the alternated choice direction. The first phase of shaping is complete when the rat makes three or less back-edge errors over two consecutive days. The second phase of shaping requires the rat to alternate its direction choices across trials. When the task starts, all gates are opened. The rat receives top/bottom and center runway rewards if it alternates its direction choice across trials. To reach second phase criteria, the rat must have four or fewer direction choice errors. In essence, phases one and two measure errors, days, and time needed to reach criteria for spatial working memory testing.

Working Memory Testing The third and final phase of testing measures spatial working memory over extended delay periods with the same alternation task in phase two. The delay period is defined as the total time it takes the rat to exit the center holding area, make a runway direction choice, drink the water, and then return to the center holding area.

On the first day of testing, the delay periods of approximately ten or twenty seconds are randomly, yet equally assigned across the thirty trials. On days two and three of testing, the delay periods of ten and thirty and ten and forty seconds are assigned, respectively.

Euthanasia After completing the third phase of testing, rats were anesthetized with ketamine (.95 ml/kg) and xylazine (.4 ml/kg) for euthanization via a transcardial perfusion. After exposing the heart, a needle was inserted and clamped inside the aorta. Before injecting fifty milliliters of saline solution (0.9 % NaCl) through this needle, the right atrium was cut. After this first injection, fifty milliliters of 4 % buffered paraformaldehyde was injected to preserve the tissue. Brains were extracted to observe any form of electrically induced lesions and the bodies were properly disposed.

RESULTS

With an independent samples t-test, enriched rats require less time to complete one trial as compared to the tDCS groups in phases one and two (alpha level of .05). In phase two, enriched rats require fewer direction choice errors to reach criteria. Because of surgical difficulties, as described later, the tDCS group did not reach the second phase of learning. A one-way ANOVA determined no significant difference between the groups for the ten second baseline delay or across the extended working memory delays (20, 30, or 40 seconds). In essence, the effects of an enriched environment occurred in phase two, in which the enriched rats learned the task in fewer days, fewer errors, and with faster lap times.

Table 1 suggests that enriched rats need less time to complete one lap or trial, which is defined as the time taken for the rat to leave and return to the center holding area. Figure 6 provides a bar graph that displays differences in lap times between all three groups and the newly combined control group of the EE and tDCS groups, since the tDCS group did not receive stimulation and was housed in standard cages. In both phases, enriched rats completed thirty laps in less time and according to a Levene's test for measuring the equality of variance, the group of enriched rats required fewer extreme lap times to complete one trial as compared to the control rats, $p<.05$.

Table1 summarizes these results and addresses the notion that an enriched environment improves the rate at which the delayed alteration task is learned, while not improving working memory performance with extended delays. The days required to reach criteria in phases one and two is nonsignificant between the control and enriched rats. In addition, there is no significant difference in back-edge errors across phase one in order for both groups to reach phase two testing. This experiment, however, does not measure back-edge errors for phase two. In contrast, enriched rats need fewer direction choice errors to reach the final and third stage of testing. A Levene's test for the equality of variance between the two groups demonstrated that enriched rats as a group have less variability or more uniform responses for direction choice errors in phase two, $p<.05$. It is possible that this reduction in direction choice errors for enriched rats is related to the difference in lap times between the two groups. Figure 7 provides a bar graph that displays differences in direction choice error means.

Table 1 also presents the nonsignificant differences in working memory performance across the delays of twenty, thirty, and forty seconds between the groups. There is no difference in performance at the ten second delay for the control group (M=12.7, SD=1.1) and the enriched group (M=12.2, SD=.94). As expected, when the delays extend across that forty second period, performance for both the enriched and control rats equally descend in a linear manner according to a repeated measures ANOVA with a Greenhouse-Geisser correction, $F(1.23, 17.24)=5.66$, $p=.024$. This information suggests that an enriched environment does not improve working memory over extended delays nor does an enriched environment reduce the group's variability for working memory with extended delays.

DISCUSSION

With an independent samples t-test, enriched rats rats construct, is one component that makes animals successful in the environment. Working memory function is the best predictor of general intelligence. In effect, finding ways to enhance working memory could improve the processing potential of humans, a discovery which may increase the likelihood of humans making decisions that improve their well-being. Being able to hold information for longer periods without noisy disruptions, allows central processes more potential to manipulate the information. As neurodevelopmental disorders like mental retardation exhibit a reduction in intelligence and working memory functions, finding interventions to improve their processing deficits will make their own life and their caregivers' lives less strenuous.

Enriched environment experiments with rats have been studied for half a century, yet the interaction with this active lifestyle and transcranial direct current stimulation has never been addressed in the literature. Human tDCS studies significantly outnumber the few tDCS rat model studies. Studying the rat model, however, helps identify and

compare neurological changes from the stimulation within the cortex, distinguish behavioral nuances from overt behavioral tasks, and determine effects from different electrode montages. Compared to humans, rat modeled experiments present less confounding variables, since the environment activity is regulated. Though this experiment will not analyze neurological morphology like dendritic spine turnover rates, it will provide another qualitative study that can be referenced to explain how certain stimulation parameters can improve or reduce performance in a working memory task. In addition, enriched environment literature has provided improvements in spatial working memory, yet existing research has not specifically addressed the effects of an enriched environment on the delayed alternation working memory task that is used in this experiment.

When considering potential experimental outcomes and how they relate to this hypothesis, there are eight individual possibilities. If the enriched environment rats improved in working memory performance, this outcome would agree with the supported notion that an enriched environment is beneficial for frontal lobe activity associated with higher order processes needed in a delayed alternation working memory task. Specifically, the parameters of the enriched environment, like the duration of enrichment, the toys present, and the ages at which the enrichment was implemented, will contribute to the accumulating literature that enriched environment benefits develop from specific environmental factors. If no main effect is observed with an enriched environment, the enriched environment condition may need longer durations and different interactive toys. In essence, any result can support human resource interventions that promote specific forms learning through proactive interactions with an environment.

If the condition of only receiving cathodal transcranial direct current stimulation presents a main effect, it will conclude that rat tDCS subjects have improved performance in a specific delayed alternation working memory task, which would be a novel discovery. In addition, improvements with this specific task suggest that the electric field properly stimulated corresponding layers and regions that would lead to improved effects. Though the brain tissue will not be analyzed for the before mentioned neuroplasticity mechanisms, the validity of the rat tDCS model and its corresponding electric field will suggest that these beneficial stimulation parameters should be contemplated and translated to human tDCS research. The effectiveness of cathodal stimulation reaffirms the notion that working memory performance is improved with less long-term potentiation mechanisms, hence reduced signal-noise ratios. If electrical stimulation does not affect performance, it is possible that shunting, duration and focality effects from the stimulation parameters were not appropriate for the specific task. Nonetheless, both significant and non-significant results from tDCS stimulation encourage other research to consider problems and merits with this experimental design.

The interaction between an enriched environment and transcranial direct current stimulation has not been examined in the rat model. If this condition improves performance in the working memory task as compared to all of the other conditions, then this interaction supports the conceptualized notion that tDCS and an enriched environment may operate through a global connectivity device (Yuste, 2011). In broader terms, this interaction could support the theories of integrated dynamic neural fields. Nonetheless, results from all conditions and dependent variables can highlight the importance of specific neuroplasticity alterations that have the potential to identify neurodevelopmental disorders, alleviate its associated symptoms, and allow normal individuals new interventions to improve global intelligence. In essence, this novel experiment has the potential to contribute to the movement of understanding the biological signature of consciousness and global intelligence.

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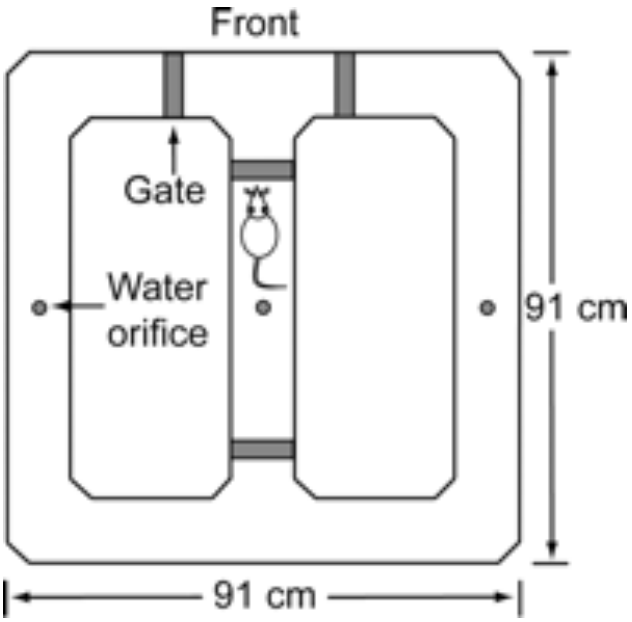

Figure 1. This delayed alternation task, commonly known as a figure-eight maze, measures reference memory and working memory.

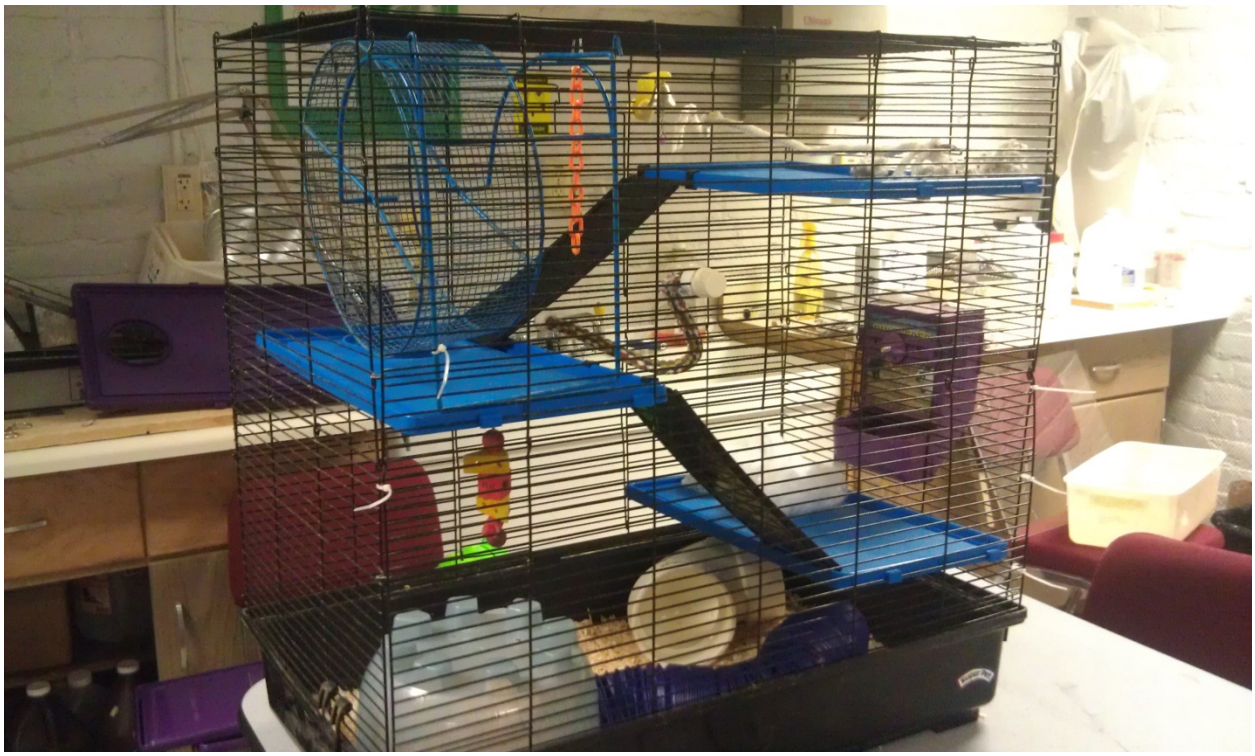

Figure 2. This enriched environment houses a running wheel with multiple toys. Toys were switched out every four days to ensure a novel environment.

Figure 3. This constant current stimulator was built by Dr. Mark Holcomb. It is designed to beep when the current is not completed. It also has a USB software device that allows for different stimulation durations and ramping effects.

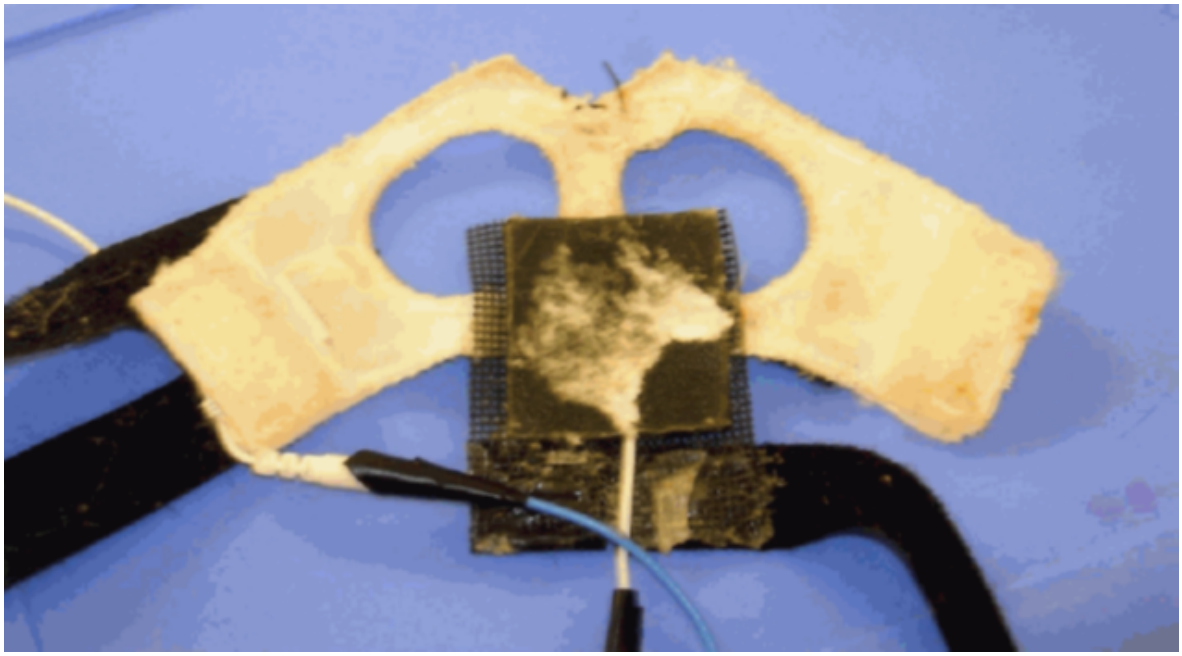

Figure 4. As modeled from Liebetanz et al. (2006), this rat jacket stabilized the cathode electrode on the rat's abdomen. Before stimulation, a conductive gel coated this electrode.

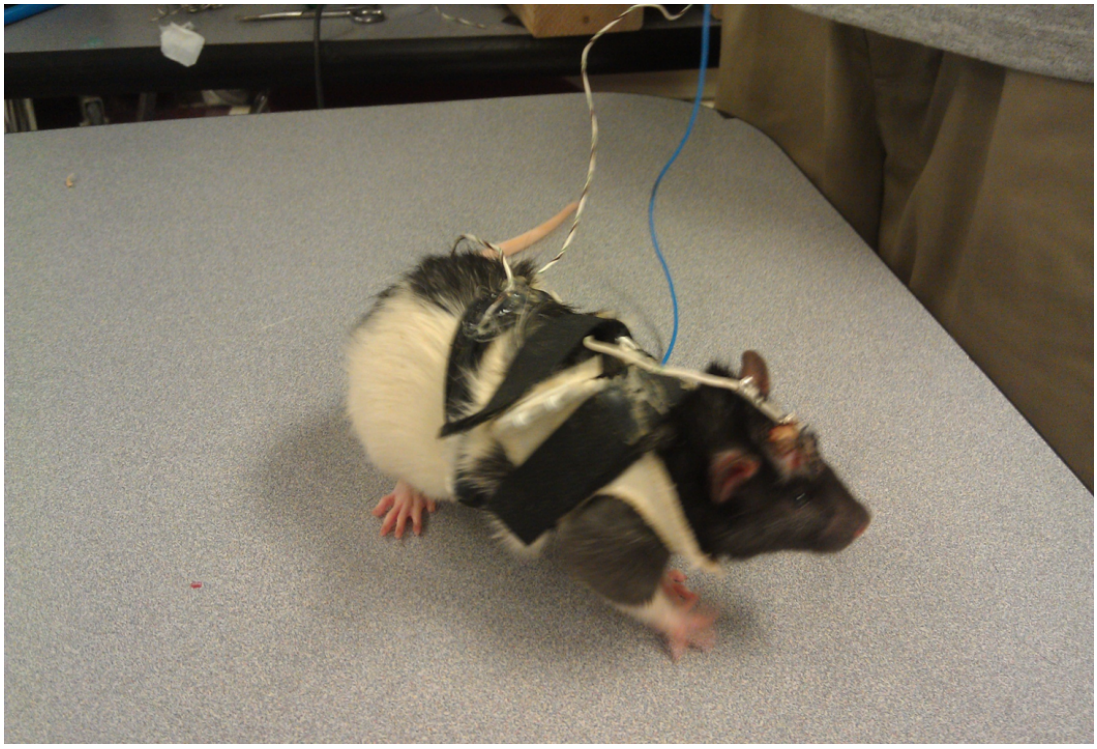

Figure 5. Though this novel surgical procedure did not successfully stabilize the electrode onto the skull, this process offers valuable insight for future tDCS studies that require a mobile rat.

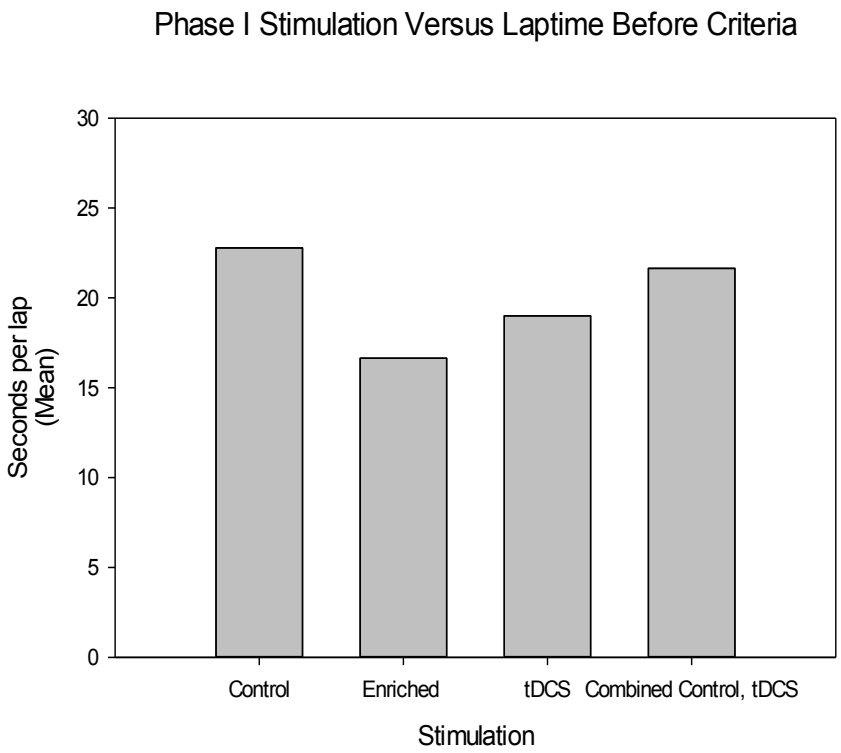

Figure 6. This graph presents differences in lap times between the groups. Since the tDCS group was not stimulated and lived in standard houses, they were combined into a new control group. This new control group and the original control group both significantly differ from the enriched group.

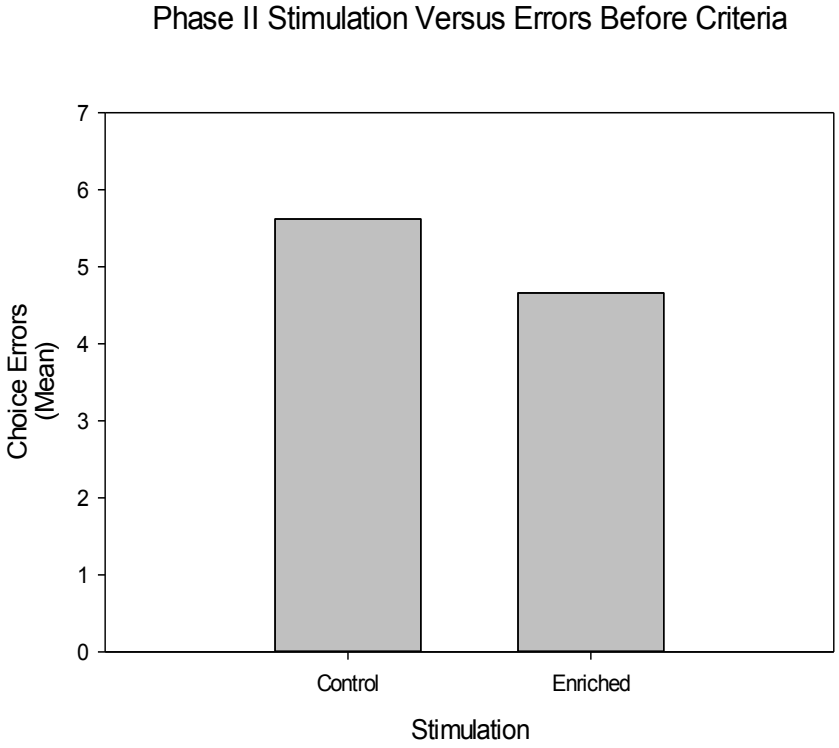

Figure 7. This graph compares the direction choice errors between the enriched and control groups.

There is a significant difference, which suggests that enriched learned the delayed alternation task with fewer errors.

Phase 1		**Phase 2**	
Days for Criteria	Control: M=4.3; SD=1.63	Days for Criteria	Control: M=7.5; SD=2.4
	EE: M=4.1; SD= .98		EE: M=6.1; SD=1.2
	t(19)=.232, p>*.05*		t(14)=1.39, p >*.05*
Lap Time (secs.)	Control: M=21.8; SD=7.4	Lap Time (secs.)	Control: M=15.1; SD=5.8
	EE: M=16.3; SD=5.8		EE: M=9.4; SD=1.7
	t(90)=3.17, p=*.002**		t(97.6)=5.08, p<*.000**
Back-edge Errors	Control: M=4.7; SD=3.1	Direction Errors	Control: M=5.8; SD=2.6
	EE: M=4.6; SD=4..4		EE: M=4.6; SD=1.9
	t(91)=.096, p>*.05*		t(88.65)=2.14, p=*.01**
Phase 3	20 Sec. Delay	30 Sec. Delay	40 Sec Delay

Control	M= 2.1; SD=1.3	M= 2.9;SD=1.6	M=3.1; SD=2.1
EE	M=1.5; SD=1.5	M=3.2; SD=1.2	M=4.0; SD=1.7
F(1.23,17.24)=.81	p>*.05*	p>*.05*	p>*.05*

Table 1. This table labels the dependent variables that support the notions that enriched rats explore more efficiently and naturally alternate direction choices with higher accuracy as compared to control rats.