The Effects of Environmental Enrichment on the Inflexible Behaviors of Autism

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The Effects of Environmental Enrichment on the Inflexible Behaviors of Autism

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ABSTRACT

Many autism research studies have been devoted to studying social interaction and the ability to communicate but relatively few have been dedicated to researching restricted and ritualistic behavior. The purpose of this study was to examine the effects of the environment on the ability to accomplish a reversal learning task, using an animal model of autism. Twelve Sprague-Dawley rats were prenatally exposed to sodium valproate (VPA), and twelve rats were prenatally exposed to saline. For each exposure group, half of the rats were housed in standard laboratory cages and the other half inhabited enriched environments, with various novel objects introduced twice weekly. All rats were trained until they acquired the behavior of turning down the right-arm of a T-maze to receive a food reinforcer. The reversal learning task occurred when the food reinforcer was placed down the left-arm of the T-maze. Latency and number of errors were recorded for each rat. Results showed that VPA rats had significantly greater latency to retrieve the reinforcer than the control group, regardless of environmental condition. Possible explanations and implications are discussed.

Introduction

Preface

Dr. Temple Grandin (2002, para.1), a prominent author and speaker on autism, recalls hearing "...a case where an autistic boy had a severe injury but he did not leave the school bus stop to get help. He had been taught to stay at the bus stop so that he would not miss the bus; he could not break that rule. Common sense would have told most people that getting help for a severe injury would be more important than missing the bus." Thus, while rules and routines are necessary to establish for everyone, it is crucial that autistic individuals learn the ability to adapt in certain situations.

Autism Spectrum Disorders, including autistic disorder, Asperger's syndrome, and Pervasive Development Disorder Not Otherwise Specified (PDD-NOS), are a group of psychological disorders, each with their own criteria for diagnosis by the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (2000). Autism Spectrum Disorders currently affect an estimated one in every 91 children (Kogan et al., 2009). Autistic disorder, also simply referred to as autism, is the most common and severe. It is a complex disorder of the nervous system, characterized by lifelong problems with social interaction, impaired ability to communicate, and patterns of repetitive behavior and restricted interests.

Out of these three domains of characteristics, relatively few studies have been dedicated to researching restricted and ritualistic behavior (Lewis & Bodfish, 1998). According to the *DSM-IV-TR* (2000), while this repetitive behavior includes stereotyped motor manners such as flapping of the hands, it can also consist of "apparently inflexible adherence to specific, nonfunctional routines or rituals" (p. 75).

This display of inflexible behavior and thinking is important to investigate due to its real-world implications. Rigid thinking and behavior not only interfere with daily activities, but also have consequences in emergency situations. Researchers use animal models of autism to study possible methods of alleviating this characteristic of inflexible behavior, as well as other attributes, of the developmental disorder.

Animal Model of Autism

Animal models of autism are usually generated by targeted brain insults or pharmacological agents. While prenatal thalidomide exposure has been linked to autism in humans, it cannot be tested in rats because thalidomide does not have the same teratologic effects in rodents (Schumacher, Terapane, Jordan, & Wilson, 1968, as cited in Rodier, Ingram, Tisdale, & Croog, 1997). However, prenatal exposure of rats to valproic acid (VPA) on the 12.5th day of gestation has been found to be an effective model, resulting in similar brain abnormalities found in autistic patients. Studies have found that the offspring of female rats injected with VPA show the following brain alterations: a reduced number of motor neurons in the trigeminal, hypoglossal, abducens, and oculomotor nuclei of cranial nerves; a significant shortening of the distance between the facial nucleus and hypoglossal, and a significant increase in the distances between anterior landmarks of the brainstem and the facial nucleus; a reduced number of cerebellar neurons; significantly fewer Purkinje cells in lobules VI-VIII and IX, but not in anterior lobes IV-V of the vermis; and reduced cerebellar nucleus interpositus, which corresponds to the globose and emboliform nuclei of the human (Rodier et al.,1997; Rodier, Ingram, Tisdale, Nelson, & Romano, 1996; Ingram, Peckham, Tisdale, & Rodier, 2000). These numerous brain abnormalities in the VPA-exposed rats are consistent with those found in numerous autopsies and brain-imaging studies of individuals diagnosed with autism.

In addition to the resemblance in brain anomalies, VPA-exposed rats also show behavioral alterations exhibited by autistic individuals. These include lower sensitivity to pain, hypersensitivity to certain non-painful stimuli, diminished acoustic prepulse inhibition, decreased number of social behaviors and increased latency to social behaviors, as well as repetitive, stereotypic-like hyperactivity and lower exploratory activity (Schneider & Przewłocki, 2005).

Environmental Effects in Non-Humans

An animal model of autism can also result from environmental and exploratory restriction. In support of this model, repetitive behaviors are often observed in zoo, farm, and laboratory animals housed in confinement. Animals that exhibit abnormal repetitive behaviors when held captive include bears, leopards, elephants, and giraffes, among countless others (Mason, Clubb, Latham, &Vickery, 2007). Not surprisingly, rodents, including Sprague-Dawley rats, display similar patterns of behavior when housed in laboratory cages.

In the late 1940's, researcher Donald O. Hebb (1949) noticed that the rats he brought home as pets for his children later showed improved problem-solving performance than their littermates who never left the laboratory. This observation pioneered research investigating the behavioral effects, as well as the effects on the brain, that occur in association with living in enriched environments. In research, environmental enrichment is defined as housing conditions that allow for enhanced sensory, cognitive, and motor stimulation relative to standard housing. Although rarely described in experimental methods, enrichment entails the presence of objects that vary in composition, shape, size, color, smell, and texture (Nithianantharajah & Hannan, 2006).

Since Hebb's (1949) behavioral observations, researchers have found that, as opposed to living in restricted environments, enriched environments have various influences on the brains of non-human animals. Diamond, Krech, and Rosenzweig (1964) found a significant increase in cortical depth in rats reared in complex environments as opposed to those housed in isolation with no stimulation. They also noted less numerous neurons, glia, and capillaries in the visual and somatosensory areas of the cortex in environmentally enriched rats compared to isolated rats. They hypothesized that the increased depth of the cortex was due to an increase in the dendritic branching between the cells and capillaries. This idea was supported by Volkmar and Greenough (1972), who found that rats reared in enriched environments had increased branching of higher-order dendrites in the visual cortex, compared to counterparts housed in isolation or pairs in standard metal cages. Since then, environmental enrichment has also been found to produce greater dendritic branching and density in the cerebellum (Floeter & Greenough, 1979), striatum (Comery, Shah, & Greenough, 1995), and hippocampus (Rampon, et al., 2000). Furthermore, Young, Lawlor, Leone, Dragunow, & During (1999) showed that environmentally enriched rats had reduced spontaneous apoptotic cell death, increased expression of neurotrophic factors, and enhanced neurogenesis compared to control rats housed in isolation.

Environmental Effects in Humans

Environmental restriction can also result in autistic-like characteristics in humans. The effects of impoverished living have been considered by studying the brains and behaviors of orphans who spend the early months and years of life in deficient conditions. Chugani et al. (2001) used neuroimaging techniques to measure the brain activity of Romanian orphans compared to controls. Prior to adoption, these children experienced dreadful circumstances, confined to cots with little interaction from caregivers and no toys for stimulation. The

researchers found significantly reduced brain activity in the orbital frontal cortex, prefrontal infralimbic cortex, amygdala, hippocampus, temporal cortex, and brain stem in a sample of these orphans after adoption. Rutter et al. (1999) worked with the English and Romanian Adoptees (ERA) Study Team, researching the differences between English and Romanian orphans adopted by English families. Of the 111 Romanian children assessed, 6% displayed quasi-autistic patterns at 4 years of age, compared to none of the orphans born and adopted within the United Kingdom. Indeed, this 6% spent significantly more time in institutionalized care before being adopted than the majority of other Romanian orphans being assessed, and they experienced worse conditions than English children. The autistic-like features of these Romanian children included social and communication deficits (i.e. lacked social reciprocity and normal social boundaries, experienced difficulty maintaining social interaction, showed limited empathy, failed to use parents for security or comfort, and used repetitive language), as well as repetitive, stereotyped behavior (i.e. intense circumscribed interests and sensory preoccupations).

Fortunately, enrichment has been shown to improve the functioning of children reared in restricted environments. Hakimi-Manesh, Mojdehi, & Tashakkori (1984) provided a sample of Iranian orphans with extra interpersonal communication and tactile, auditory, and visual stimulation for 5 minutes per day over a 6-week period. Infants receiving this extra stimulation showed significantly greater improvements on measures of mental and psychomotor development, as evaluated by the Bayley Scales of Infant Development, after 6 weeks compared to counterparts receiving no such attention. Furthermore, 6 months after treatment ceased, the enriched group displayed no drop in scores of mental or psychomotor development, whereas the control group showed a significant drop in measures of psychomotor development. Thus,

enrichment can have significant, lasting effects on the functioning of humans exposed to restricted environments.

Present Investigation

The concept of environmental enrichment relates to autism in the following way: if an impoverished environment engenders this class of behaviors seen in autism, providing an enriched environment should ameliorate the ritualistic, inflexible behaviors seen in autism. As noted by Lewis, Tanimura, Lee, and Bodfish (2007), environmental restriction may, initially, seem to have limited relevance to autism. However, individuals with autism suffer from deficits in social, emotional, motor, and cognitive functioning, making them functionally environmentally restricted. Thus, the development of strategies for bringing individuals with autism into more functional or meaningful contact with their environment may have significant clinical implications for preventing repetitive and inflexible behaviors in autistic individuals.

The purpose of this study is to examine the effects of the environment on learning a reversal learning task, using an animal model of autism. The study investigates the following hypotheses: 1) rats prenatally exposed to VPA who are housed in an enriched environment will accomplish the reversal learning task more quickly and accurately than their counterparts housed in an impoverished environment, 2) control rats housed in an enriched environment will learn the task more quickly and accurately than rats prenatally exposed to VPA living in the same environment, and 3) the effects of environmental enrichment will override the effects of the valproic acid, causing the environmentally enriched VPA rats to perform more quickly and accurately than control rats housed in impoverished environments.

Materials and Methods

All procedures were reviewed and approved by the Animal Review Committee at Allegheny College and performed in accordance with the guidelines set forth in the NIH Guide for the Care and Use of Laboratory Animals.

Subject Population: Four timed-pregnancy female rats were ordered from Hilltop Lab Animals, Inc. and arrived at Allegheny College on day two of gestation. Their offspring resulted in 22 control and 28 experimental rats. Only 12 control and 12 experimental rats were used in behavioral testing.

Apparatus: A reversal learning task was performed in a T-maze (Figure 1). A holding chamber (24.1 x 14.0 x 14.0 cm) was offset from the arms of the maze by a metal gate, which the experimenter could manually operate with a pulley. Both arms of the maze were identical, measuring 45.7 x 20.3 x 25.4 cm each.

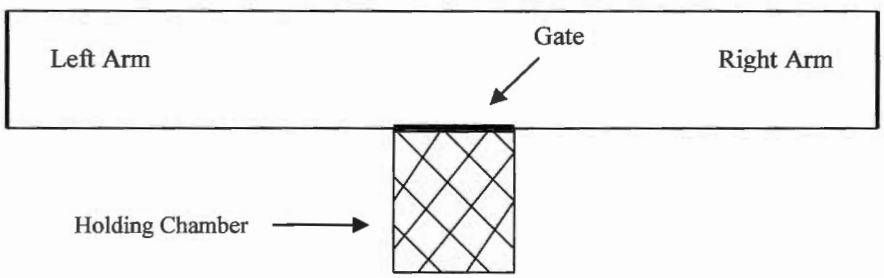


Figure 1: T-maze used for behavioral testing.

Procedure:

Drug Administration

Four dams were housed together in a standard laboratory cage (42.0 x 22.0 x 20.0 cm), where food and water were available *ad libitum*. On day 12.5 post-conception, two of the dams received a single intraperitoneal injection of 400 mg/kg sodium valproate. Their offspring resulted in the experimental group. The other two dams received single intraperitoneal injections

of saline of the same volume. Their offspring resulted in the control group. The four dams were returned to their cage, but separated on day 17 post-conception to prepare for birth.

Housing Environments

Of the two dams injected with VPA, one was placed in an impoverished environment (IE) while the other was housed in an enriched environment (EE). The same living conditions were assigned for the two control dams to form a total of four conditions: experimental IE, control IE, experimental EE, and control EE.

Impoverished environments consisted of opaque standard cages (42.0 x 22.0 x 20.0 cm), located in the animal colony (See Appendix A, Figure 1). The colony had 24-hour lighting and background music. Woodchips provided bedding. Food and water were available *ad libitum*, except prior to behavioral testing.

Enriched environments consisted of clear, rectangular aquariums (50.5 x 26.0 x 27.0 cm). Various novel objects were introduced twice weekly. These included rubber balls, wooden blocks, plastic toys, plastic balls with bells inside, running wheels, and tunnels (See Appendix A, Figure 2). Objects consisted of a variety of colors, shapes, and textures. Again, woodchips provided bedding, and food and water were available *ad libitum*, except prior to behavioral testing.

The offspring were born into one of these assigned environments and weaned on postnatal day (PND) 28. Each litter was separated by sex on PND 38. On PND 44, the rats were further divided so that only 3-4 rats of the same sex occupied each cage. IE rats were housed in opaque standard cages in the colony. EE rats were housed in clear cages (54.6 x 30.5 x 20.3 cm) with novel objects and moved to the colony. Six rats from each of the four groups were used for

behavioral testing, with four males and two females per group. Cages were cleaned twice weekly throughout the experiment.

Behavioral Training and Testing

After approximately 80 days of being housed in their appointed environments, rats began training in the T-maze. Prior to each training session, they were deprived of food for 23 hours. Once placed in the T-maze, rats were trained by successive approximations to turn down the right-arm of the maze to retrieve a piece of Kellogg's® Froot Loops® cereal. During this phase of training, each rat completed 10 trials with the left-arm blocked off with a piece of cardboard. The piece of cardboard was then removed to expose both arms of the maze. Training continued with the Froot Loops® placed at the end of the right-arm, except for one VPA-EE male and one control-IE male who would not respond to this reinforcer. These rats were given a piece of LabDiet 5P00 RMH 3000 food pellet, the same used throughout the colony, in place of cereal. A timer was started once the gate to the T-maze was opened at the beginning of each trial. If the rat turned down the correct arm, the gate was held open by the experimenter while they retrieved their food reinforcer. However, if the rat made an incorrect turn, the gate to the holding chamber was closed so that re-entering the holding chamber would not be counted as an error. The gate remained closed until the rat retrieved the reinforcer. Time to retrieve the food reinforcer (latency) was recorded for each subject, as well as the number of wrong turns (errors). Each rat completed a minimum of 10 and a maximum of 12 trials on any day of testing. All rats continued training until they made seven or more correct responses for three consecutive testing days (Tanimura, Yang, & Lewis, 2008).

Once rats met the criterion, the food reinforcer was placed at the end of the left-arm of the T-maze, designating the reversal learning task. Each rat completed one trial under this condition. Latency and number of errors were recorded for each rat and analyzed.

Results

The dependent variables, latency and number of errors, were each analyzed by a Two-Way Between Subjects ANOVA. Homogeneity of variance was met for both variables, and significant differences were determined using an alpha level of 0.05.

Latency

There was a main effect of group, $F(1, 20) = 9.377$, $p = .006$. VPA rats showed significantly increased latency ($M = 53.583$; +/- 7.824 *SEM*) compared to the control group ($M = 27.250$; +/- 3.906 *SEM*), regardless of their environmental condition (Figure 2).

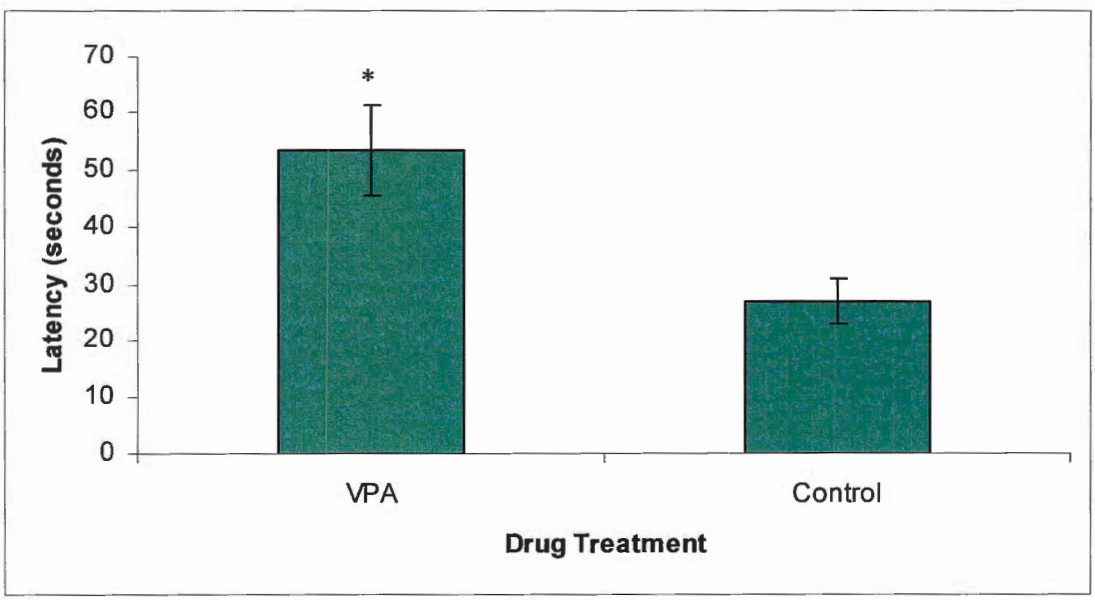


Figure 2: VPA rats ($M = 53.583$) exhibited significantly increased latency to retrieve the food reinforcer, as compared to their control counterparts ($M = 27.250$). Data expressed as group mean +/- *SEM*, $*p < .05$.

There was no significant difference in latency between subjects housed in EE ($M = 45.583$; +/- 8.227 *SEM*) and IE ($M = 35.250$; +/- 5.954 *SEM*), $F(1, 20) = 1.444$, $p = .244$ (Figure 3).

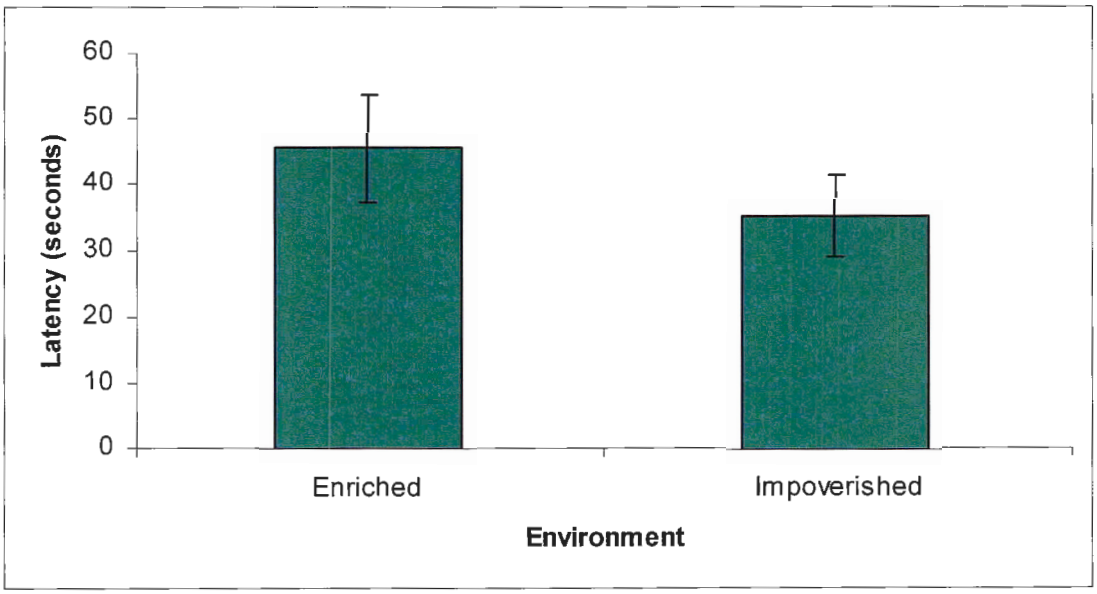


Figure 3: Latency for environmentally enriched rats ($M = 45.583$) was slightly higher than that of impoverished rats ($M = 35.250$), but the difference was not significant. Data expressed as group mean +/- *SEM*, $p < .05$.

There was no significant interaction between group (VPA or control) and environment, $F(1, 20) = 1.308$, $p = .266$ (Figure 4).

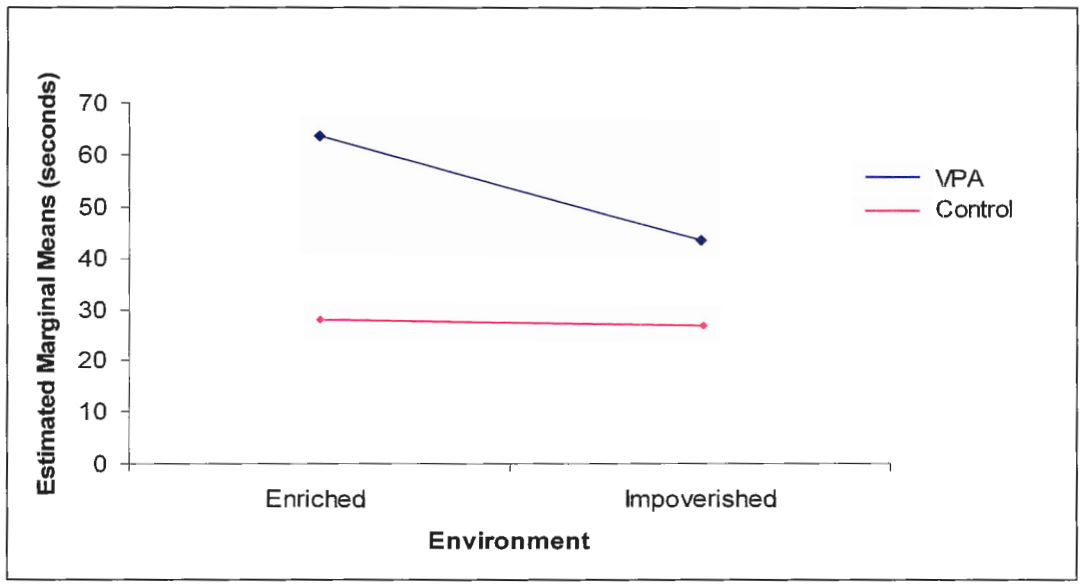


Figure 4: There was no significant interaction between group and environment. VPA rats (EE $M = 63.667$, IE $M = 43.500$) showed greater latency than the control group (EE $M = 27.500$, IE $M = 27.000$) regardless of rearing environment.

Errors

There were no main effects or interaction for number of errors. Results are presented as follows: Average number of errors between VPA ($M = 1.000$; +/- .174 *SEM*) and control rats ($M = .583$; +/- .193 *SEM*) did not differ significantly, $F(1, 20) = 2.358$, $p = .140$ (Figure 5).

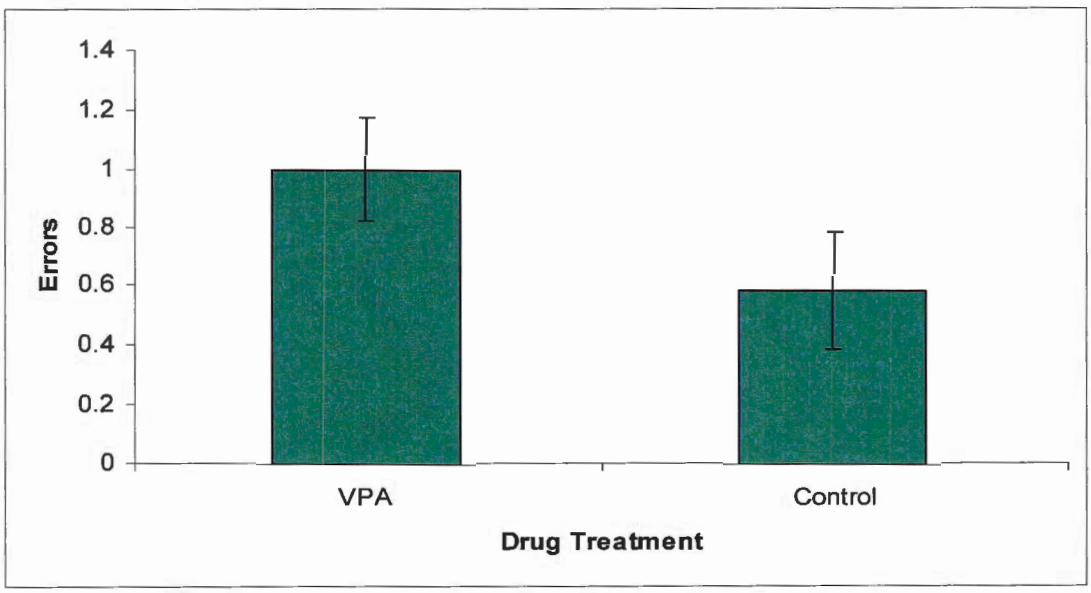


Figure 5: VPA rats ($M = 1.000$) committed a higher average number of errors than the control group ($M = .583$), but the difference was not significant. Data expressed as group mean +/- *SEM*, $p < .05$.

As with latency, number of errors was not affected by environmental condition. EE (M = .833; +/- .207 *SEM*), IE (M = .750; +/- .179 *SEM*), $F(1, 20) = .094$, $p = .762$ (Figure 6).

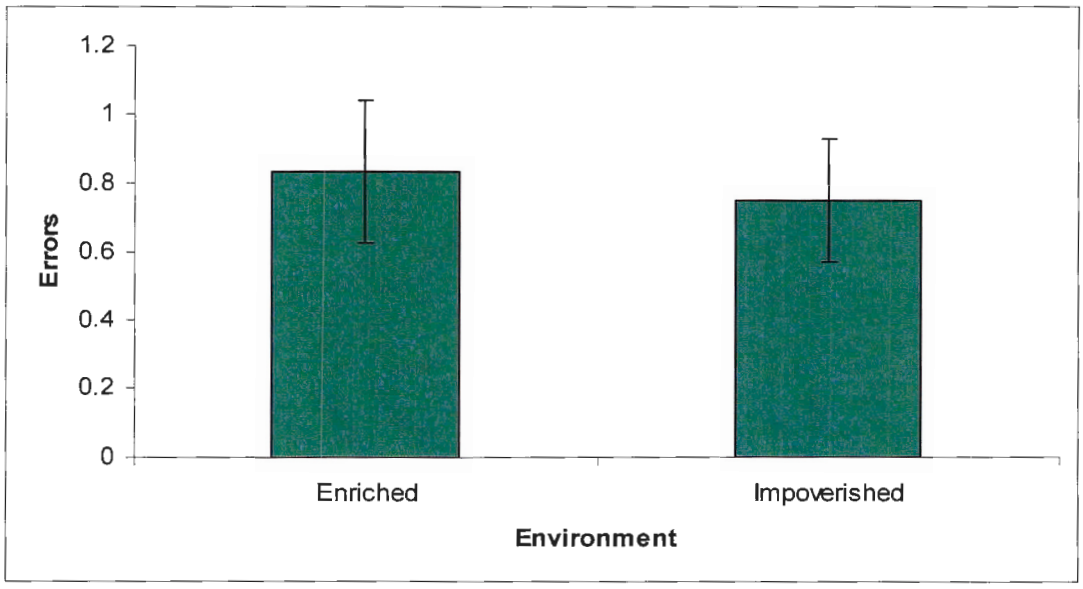


Figure 6: Environmentally enriched rats (M = .833) committed a slightly higher average number of errors than rats in impoverished environments (M = .750), but the difference was not significant. Data expressed as group mean +/- *SEM*, $p < .05$.

Finally, there was no significant interaction between group and environment, $F(1, 20) = .094$, $p = .762$ (Figure 7).

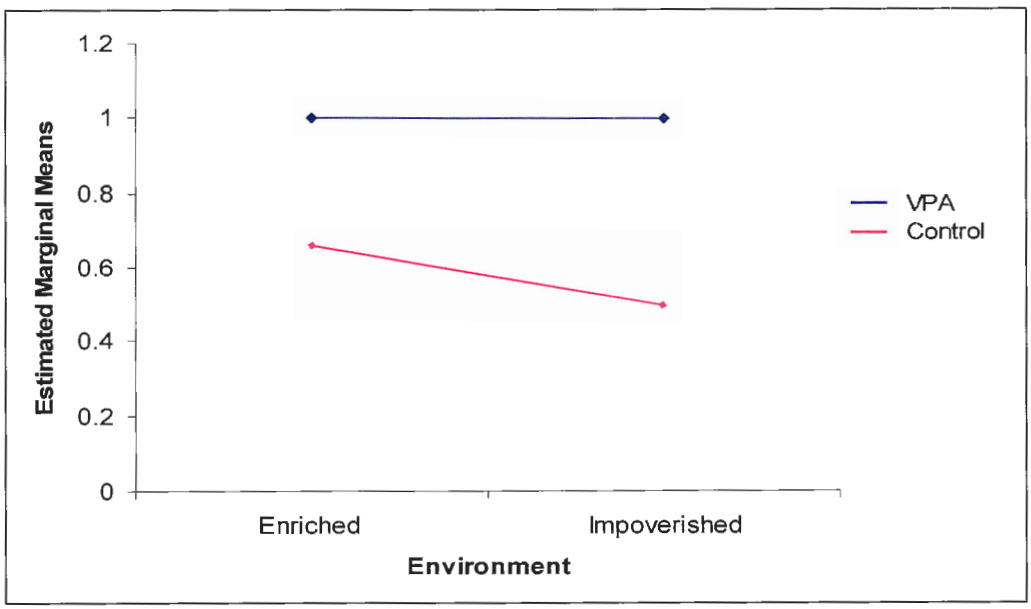


Figure 7: There was no significant interaction between group and environment. VPA rats (EE $M = 1.000$, IE $M = 1.000$) displayed a greater number of errors than the control group (EE $M = .667$, IE $M = .500$) despite differential housing environments, though the difference was not significant.

Discussion

VPA rats displayed significantly increased latency compared to control subjects, regardless of environment. There was no significant difference in number of errors between VPA and control rats, or IE and EE rats. Therefore, the enriched environment, as was constructed for the present experiment, did not appear to alter performance. Similarly, impoverished living had no detrimental effect on latency or number of errors. One explanation for the lack of environmental effects is the component of social variation. Most of the aforementioned research citing brain alterations in animals due to differential housing included differences in physical complexity, as well as social enrichment between environments. Enriched environments housed 6 to 12 animals, whereas impoverished environments had animals housed in isolation (Comery,

Shah, & Greenough, 1995; Diamond, Krech, & Rosenzweig, 1964; Floeter & Greenough, 1979; Volkmar & Greenough, 1972; Young, Lawlor, Leone, Gragunow, & During, 1999). However, these studies were investigating the effects of environmental enrichment on animals that did not represent an autistic model, so they could be expected to benefit from social enrichment. The present study investigated only the effects of physical enrichment, through the introduction of various novel objects, because of the social deficits associated with autism. For this reason, the number of littermates in both environments was controlled at two to three per cage. The design of this experiment was created under the assumption that providing social enrichment may not be effective in treating autistic individuals in the real world. The communication and social impairments of people diagnosed with autism may prevent them from engaging in social behaviors, even when the opportunity is presented.

Likewise, it could be inferred that autistic individuals may not be apt to explore a physically enriched environment either. Indeed, Pierce and Courchesne (2001) found that, when given the opportunity to play and explore in a room full of novel objects, children with autism spent significantly less time engaging in exploratory behaviors than children without autism. While control children spent 88% of their time exploring the objects, autistic children spent only 42% of their time engaging in active exploration. In addition, they displayed significantly more repetitive movements and explored fewer objects overall. This may explain why environmental enrichment had no significant effect on VPA rats in the current study; that is, just because they were presented with various novel objects does not ensure that they will engage in exploratory behavior.

Another explanation for the lack of significant difference in latency and errors between the EE and IE conditions is that the training itself could theoretically be considered enrichment.

Thus, EE and IE control rats performed similarly, as did EE and IE VPA rats, because the IE subjects were not necessarily impoverished. Although these rats were not presented with novel objects and toys, they experienced the same amount of training in the T-maze. Training for each rat sometimes occurred as often as twice per week, with each session lasting up to one hour. In this sense, IE rats were not entirely restricted. This seems probable, since IE control rats performed just as well as their EE counterparts, who had no hindrance in exploratory behavior like VPA rats. Hence, while environmental enrichment benefited control rats, the training probably helped IE control subjects just as much. The same effect could have occurred with VPA subjects as well.

Although EE and IE subjects performed similarly, EE rats as a group still had slightly higher latency times and committed a greater number of errors. Although these differences in performance between EE and IE rats were not significant, they are worth investigating. It is possible that the latency and error data were, in fact, somewhat compromised by the rats' exploratory behavior. For example, during the reversal task, it was noted that one VPA EE female rat immediately exited the holding chamber and turned down the correct (left) arm of the maze; but instead of retrieving the food reinforcer, she turned around and proceeded down the opposite arm. The rat continued to traverse back and forth, resulting in two errors and a latency time of 81 seconds. Therefore, what appears as worsened performance may actually be a result of overall increased exploratory behavior. In this case, increased latency and errors should be viewed as favorable, as more flexible behavior was the goal of enrichment.

One must be careful when trying to draw conclusions from this data as to why EE and IE rats performed so similarly. There is no way of telling how much EE rats engaged in their environments or how much IE rats benefited from training. Future studies should observe EE rats

in their environments to calculate how much time was spent in exploration. Also, using another task that measures flexible behavior, but does not require as much training for IE rats, should be considered. Taking both of these measures may help to better assess the true reason for EE rats performing slightly better, though not significantly.

There are several limitations to this study. Foremost, the subjects were rats, not humans. Therefore, the real-world implications of environmental enrichment for autistic individuals cannot be entirely inferred from the present results. However, using the animal model of autism provides a good starting point from which to direct further research. Also, due to time constraints, only six rats per group (24 total) could be trained for the reversal learning task. Using a larger number of subjects may yield more comprehensive results.

As already mentioned, the effects of social interaction were not considered. Future investigations should examine the role of social enrichment versus physical enrichment by comparing the behaviors of subjects housed in environments composed of solely social enrichment, solely physical enrichment, and a combination of both. The effects of impoverished rats housed in isolation should also be considered.

Additionally, because the time and quality of exploratory behavior of novel objects was not observed, the true effect of enrichment could not be assessed. It would be beneficial for future studies to perhaps expose rats to novel objects and situations for only a certain period of time each day and record the time spent engaged in actual exploration, as well as the quality of engagement.

In conclusion, due to repetitive behaviors and restricted interests, autistic individuals may spend less time exploring their environments. These characteristic manners can inhibit the acquisition of flexible behavior. Although physically complex environments were not found to

benefit VPA rats in the present experiment, future research is needed to find other possible methods of encouraging more flexible, and discouraging non-functional, behavior.

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Appendix A



Figure 1: An example of rats in an impoverished environment.



Figure 2: An example of rats in an enriched environment.