The VPA Model of Autism and Effects on Avoidance Learning in Adult Male Sprague-Dawley Rats

A Senior Comprehensive Project
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Abstract

Autism is a neuro-developmental disorder characterized by socio-behavioral impairments, including impairments in social interaction, communication deficits, and patterns of restricted and repetitive behavior. Recently, an animal model of autism induced by prenatal exposure to valproic acid (VPA) has been proposed as a way to study features of autism that cannot otherwise be studied in humans. The present study utilized the VPA model to examine differences in novel/familiar object preference, as well as performance on a two-way active avoidance learning task among VPA and control rats. VPA rats showed a significant preference for familiar objects, while controls showed a significant preference for novel objects. On the avoidance learning procedure, VPA rats exhibited lower numbers of both escapes and avoidances compared to controls. Additionally, although the results were not statistically significant, a trend showed that the number of avoidances for the control rats increased at a higher daily rate than for the VPA rats.

The VPA Model of Autism and Effects on Avoidance Learning in Adult Male Sprague-Dawley Rats

What is autism?

Autism spectrum disorder (ASD) is a neuro-developmental disorder that typically manifests in early childhood. It is characterized by several socio-behavioral impairments, including impairments in social interaction, both verbal and nonverbal communication, and restricted, repetitive, and stereotyped patterns of behavior, interests, and activities (American Psychiatric Association, 1994).

The word autism comes from the Greek word "autos," meaning self. First used by Eugene Bleuler in 1911, the term originally referred to a condition in which fantasy dominated over reality, and was originally regarded as a symptom of schizophrenia and other disorders. (Bleuler, 1911).

In the early 1940s, "autism" was first used in its modern sense, as researchers first began to use the term to describe children with various emotional or social problems. In 1943, Leo Kanner described his study of 11 children who he collectively described as "mostly aloof, with limited, bizarre or absent communication, and absorbed by stereotypical movements and interests" (Kanner, 1943). Kanner eventually coined the term "early infantile autism" to describe these children as having a pattern of behavior characterized by limited social interaction, repetitive behavior, communication impairment, and high resistance to change (Kanner, 1949). However, up until the 1960s, there was little awareness of autism, and even then it was still widely regarded as a form of "childhood schizophrenia," with emotionally cold parenting as the cause. It wasn't until the Diagnostic and Statistical Manual of Mental Disorders (DSM)-III was

released in 1980, that the American Psychiatric Association finally distinguished autism from childhood schizophrenia. This continued to evolve further, and in 1987, the DSM-IIIR introduced a checklist of criteria to aid in diagnosing autism, and both the DSM-IV and DSM-IV-TR continued to expand the definition of autism until "autism spectrum disorders" included Autistic Disorder, Asperger's Disorder, and Pervasive Developmental Disorder Not Otherwise Specified (PPD-NOS) (American Psychiatric Assoiciation, 1987, 1994, 2000). The DSM-V, released in 2013, eliminated these categories in favor of a single diagnosis of Autism Spectrum Disorder (ASD) with three possible levels of severity (American Psychiatric Association, 2013).

For decades after Kanner's original publication, childhood autism was considered to be a rare condition with a prevalence of approximately 2-4 per 10,000 children (Wing & Potter, 2002). However, more recent estimates suggest that these rates have risen to as high as 110 per 10,000, with odds of diagnosis four times higher in boys than in girls (Kogan et al., 2009). The ever changing diagnostic critera for ASD is the most commonly cited factor for this increase in prevalence (Matson & Kozlowski, 2011). Reported rates of ASD have been growing steadily for years, and it appears that this trend will continue (Hertz-Picciotto & Delwiche, 2009).

While the complete etiology of autism is not known, it does have a strong genetic component (Folstein & Piven, 1991), and an exposure to at least three different teratogens appears to be a risk factor for the disorder: ethanol (Nanson, 1992), thalidomide (Stromland et al, 1994), and valproic acid (VPA) (Christianson et al, 1994).

Valproic Acid

Valproic acid (VPA) is a Food and Drug Administration (FDA) approved prescription anticonvulsant, used to treat certain types of seizures. It is also prescribed to treat manic or

mixed episodes in people with manic-depressive (bipolar) disorder, and is sometimes used to prevent migraines. Valproate products are also commonly used off-label for uses unapproved by the FDA, such as the treatment of other psychiatric conditions (FDA, 2013; National Institutes of Health, 2014).

Valproic acid is a known human teratogen, and exposure during pregnancy is associated with a three-fold increase in the rate of major anomalies, including spina bifida, anencephaly, cardiac, craniofacial, skeletal, and limb defects, and others (Ornoy, 2009). Valproate sodium, valproic acid, and divalproex sodium all carry a "D" rating from the FDA for the treatment of seizures, meaning that the potential benefit of the drug in pregnant women may be acceptable despite its potential risks. However, this "D" rating was recently changed to an "X" rating for migraine prevention, meaning the risk of use in pregnant women clearly outweighs any possible benefit of the drug (FDA, 2013).

Recent epidemiological investigations have also identified a link between prenatal exposure to VPA and an increased risk of autism spectrum disorder (Bromley et al., 2013; Christensen et al., 2013). By examining the phenotypic abnormalities of the face and developmental disabilities in children who were exposed in utero to VPA, a clinical link between VPA and autism has been recognized (Christianson et al., 1994). Further, a large population-based study consisting of a sample of 655,615 children born in Denmark from 1996 through 2006 has provided the strongest relationship between VPA and ASD to date (Christensen et al., 2013). In their analysis, they controlled for other known risk factors of ASD and potential confounders such as parental psychiatric disease, parental age at conception, congenital malformations, and maternal epilepsy. Outcomes were divided as Autism Spectrum Disorder

(ASD) or childhood autism (the most severe diagnosis), and absolute risks for each were reported. The absolute risks for all children for ASD and childhood autism were 1.53% and 0.48%, respectively, while the risks were 4.42% and 2.5% for children exposed to VPA in utero (Christensen et al., 2013).

The VPA Model of Autism

Because of the link between prenatal VPA exposure and increased prevalence of ASD in humans, an animal model of autism induced by prenatal exposure to VPA has been proposed as a way to study features of autism that cannot be studied in humans. In this model, pregnant dams receive a single intraperitoneal injection of VPA on the 12.5th day of gestation, ranging in dosage from 350-600 mg/kg (Rodier et al., 1997). Offspring of female rats injected with VPA on the 12.5th day of gestation have been shown to exhibit several brain stem and cerebellar abnormalities resembling those found at autopsy and in brain imaging studies of autistic patients (Ingram et al., 1999, Rodier et al., 1997, Hashimoto et al., 1995). Furthermore, these offspring have been shown to display obvious behavioral symptoms associated with autism, including lower sensitivity to pain, diminished acoustic prepulse inhibition, repetitive/stereotypic-like hyperactivity, and decreased number of social behaviors as well as increased latency to social behaviors (Schneider & Przewlocki, 2005). This provides a rationale for a pharmacological animal model of autism using prenatal exposure to neurotoxicants such as VPA (Murcia et al., 2005), and therefore, by utilizing the VPA animal model of autism, researchers are able to carry out complex developmental, behavioral, and immunological analyses that are not possible in humans.

Learning and Autism

There are many similarities between ASD and learning disabilities. For example, both generally begin early in life, both impact communication and social interaction, and both have a strong genetic basis and are often associated with other neurological conditions (Cox & Mesibov, 1995). Autism and learning disabilities are co-associated, and the two are so closely linked that there has even been some debate regarding whether or not they can be viewed as distinct syndromes (O'Brien & Pearson, 2004). In the general population, the prevalence of learning disabilities is approximately five percent (Lyon, 1996). However, in a review of 23 studies of the epidemiology of autism from 1966 through 1998, it was found that 19.4 percent of patients with ASD functioned within the normal range of intelligence, 29.4 percent had mild to moderate learning disability, and 41.9 percent had severe to profound learning disability (Fombonne, 1999).

Because of the co-association of ASD and learning disabilities, the VPA model of autism has often been used to study learning and memory. Previous studies have examined various types of learning in VPA rats (Bambini-Junior et al., 2011, Sui & Chen, 2012), but avoidance learning has not yet been studied. The present study utilizes the VPA model of autism to examine performance on a two-way active avoidance learning procedure.

Avoidance learning is defined as "a type of instrumental or operant learning in which the subject must learn to make some response to avoid a noxious or aversive stimulus" (Penguin Dictionary of Psychology, 2009). In this case, the reinforcement for the avoidance behavior is to not experience the aversive stimulus. Essentially, the reinforcement is the absence of punishment. In real life, avoidance learning has many practical applications. For example, if a

child touches a hot stove, they associate touching the stove with the pain of being burned, and avoid touching the stove in the future.

Hypothesis

The present study examined the performance of adult male Sprague Dawley rats prenatally exposed to valproic acid on an object preference test, as well as a five-day active avoidance learning procedure. Rats prenatally exposed to valproic acid were expected to show preference for a familiar object, while control rats were expected to show preference for a novel object. In turn, the VPA rats were expected to spend more time on the side of the testing chamber containing the familiar object, while control rats were expected to spend more time on the side of the testing chamber containing the novel object. On the avoidance learning procedure, it was hypothesized that the VPA rats would exhibit a slower rate of learning, and thus have a lower number of escapes and avoidances overall when compared to the controls.

Method

Animal Subjects

Female Sprague Dawley rats from the local breeding colony were housed individually with a 24 hour light cycle, controlled temperature (24 ± 1° C), continuous exposure to FM radio, and access to food and water ad libitum. The rats were mated and pregnancy was determined by the presence of a vaginal plug on embryonic day 1 (E1). For the VPA group, valproic acid was dissolved in saline for a concentration of 360 mg/ml. Pregnant dams in the VPA group received a single intraperitoneal injection of 470 mg/kg of the VPA solution on embryonic day 12.5, and dams in the control group received no exposure to VPA. After giving birth, dams were allowed

to raise their own litters until weaning on postnatal day 21. After weaning, rats were housed socially in groups of 3-4. Control rats and VPA rats were housed in separate cages. Due to previously described gender differences within the VPA model of autism (Schneider et al., 2008), female rats were not included in this study. The VPA group consisted of nine adult male Sprague Dawley rats, and the control group consisted of five, all ranging in age from 180-240 days.

Materials

Object Preference Test

The object preference test was conducted using novel and familiar plastic toys, an opaque plastic cage, and two stopwatches for timing. The toys varied in terms of shape and color, but all were made of hard plastic and all measured approximately 4.5 x 6.5 centimeters. The cage measured 30.5 x 56 centimeters and was divided equally in half by a mid-line marker.

Avoidance Learning

The avoidance learning procedure was conducted using Columbus Instruments' Passive/Active Avoidance System model PACS-30. The cage is constructed of stainless steel and its dimensions are 22.86 x 27.6225 x 48.26 centimeters. The avoidance enclosure is divided into two compartments separated by a 6.6675 x 7.62 centimeter opening, and the floors are constructed with steel bars spaced 1.905 centimeters apart, and capable of giving electrical shocks ranging from 0.0 mA to 1.0 mA in 0.1 mA increments. The system is also equipped with lights and a speaker, which can be programmed for use as a conditioned stimulus. The light intensity can be adjusted from 0 to 150 Lux and duration can range from 00:00 to 59:59. The

sound stimulus frequency can be adjusted from 0.2 to 13.0 KHz in 100 Hz increments, the intensity can range from mute to 115 dB, and the duration can range from 00:00 to 59:59.

Procedure

Object Preference Test

An object preference test was completed in order to screen rats for autistic-like symptoms. The day before object preference testing, a shiny silver plastic toy was placed into the rat cage for investigation. After 24 hours, the toy was removed. In an opaque plastic cage, the familiar silver toy was placed on one side, and a novel, similarly sized, but differently shaped shiny red plastic toy was placed on the other side. The cage was divided equally in half, and the rats were introduced to the cage on the mid-line marker. Trials were one minute long, and timing began when rats were placed on the mid-line marker. The frequency of times each object was investigated, as well as amount of time spent on familiar versus novel sides of the cage were measured. Investigation frequency was defined as the number of times the rat approached and interacted with the object with its head and forelimbs, and side preference was the amount of time spent on either side of the testing chamber. A rat was considered to be on one side of the testing chamber when its head and forelimbs crossed the mid-line marker. A preference ratio was computed using the following formula: (familiar investigation – novel investigation) / (familiar investigation + novel investigation). The ratio spans from +1 to -1, indicating the ratio of time spent in investigation of the familiar stimulus (+1) versus the novel stimulus (-1). The formula was also used to compute ratios for amount of time spent on each side of the testing chamber.

Avoidance Learning

Two-way active avoidance learning was conducted in an automated shuttle box. Rats underwent a previously described avoidance-learning procedure (Gruss et al., 2010) in which they were exposed to 50 trials daily for five consecutive days. At the beginning of each of the five testing days, the rats were allowed a three minute habituation period to explore the shuttle box before testing began. Each trial began with the conditioned stimulus, a 2.4 kHz tone presented for a maximum duration of 5 seconds. This was immediately followed by the unconditioned stimulus, a 0.1 mA foot-shock for a maximum 10 second duration. If the rat moved to the opposite compartment during the conditioned stimulus but prior to the unconditioned stimulus (i.e. within the first 5 seconds), an avoidance response was recorded and the shock was not administered. If the rat moved to the opposite compartment during the unconditioned stimulus (i.e. after the first 5 seconds but within the first 15 seconds), the shock was terminated and an escape response was recorded. If the rat failed to move to the opposite compartment within the total allotted 15 seconds, a failure was recorded. After each trial was completed, the inter-trial interval of 15 seconds started. This procedure was repeated a total of 50 times daily for each rat. The shuttle box was cleaned with 10% alcohol before each animal was tested in order to remove possible odor cues. In order to avoid potential circadian variations in performance, the order in which rats were tested was varied each day. If rats failed to show adequate signs of learning after two days of trials, they were removed from the study in order to avoid potential bodily harm and/or signs of learned helplessness. However, in this case, no rats were removed from the study.

Results

Object Preference

The object preference ratio of the VPA group (M=0.323, SD=0.099) was significantly greater than the object preference ratio of the control group (M=-0.395, SD=0.04), $t(12)=15.324$, $p<0.001$, which shows that the VPA rats showed a strong preference for the familiar object, while the control rats showed a strong preference for the novel object.

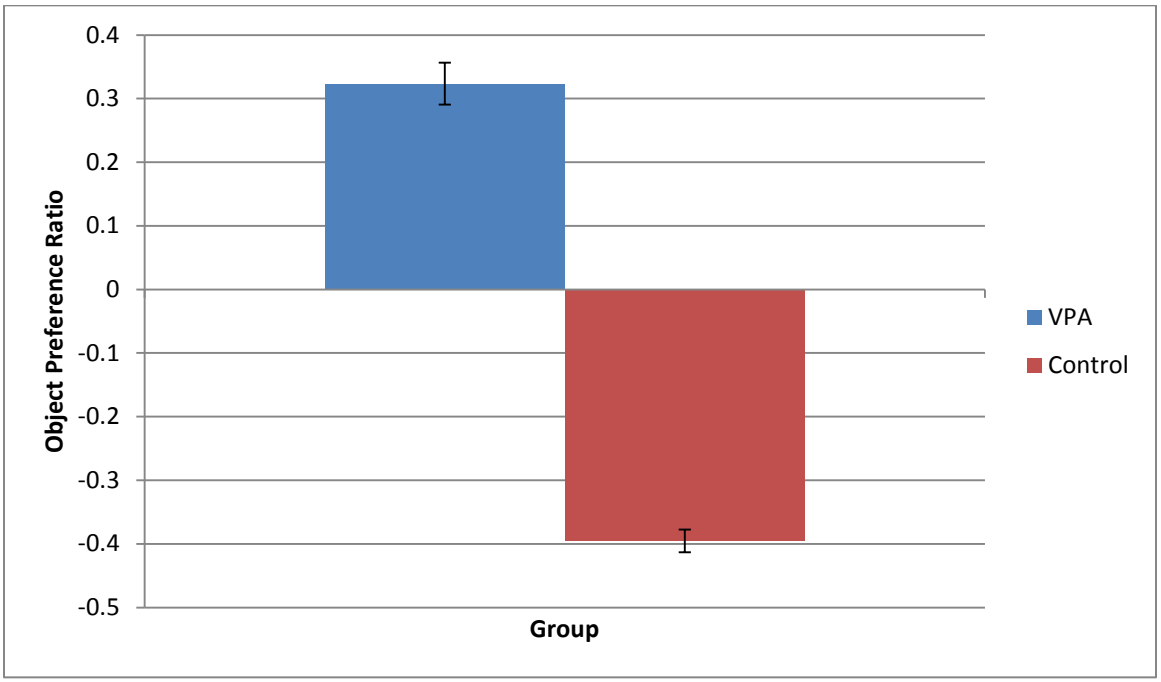


Figure 1: Graph showing object preference ratios for the VPA rats (n=9) and control rats (n=5). Error bars show standard error of the mean.

Side Preference

The side preference ratio of the VPA group (M=0.298, SD=0.111) was significantly greater than the side preference ratio of the control group (M=-0.347, SD=0.157), $t(12)=9.015$, $p<0.001$, which shows that the VPA rats spent significantly more time on the side of the cage containing the familiar object, while control rats spent significantly more time on the side of the cage containing the novel object.

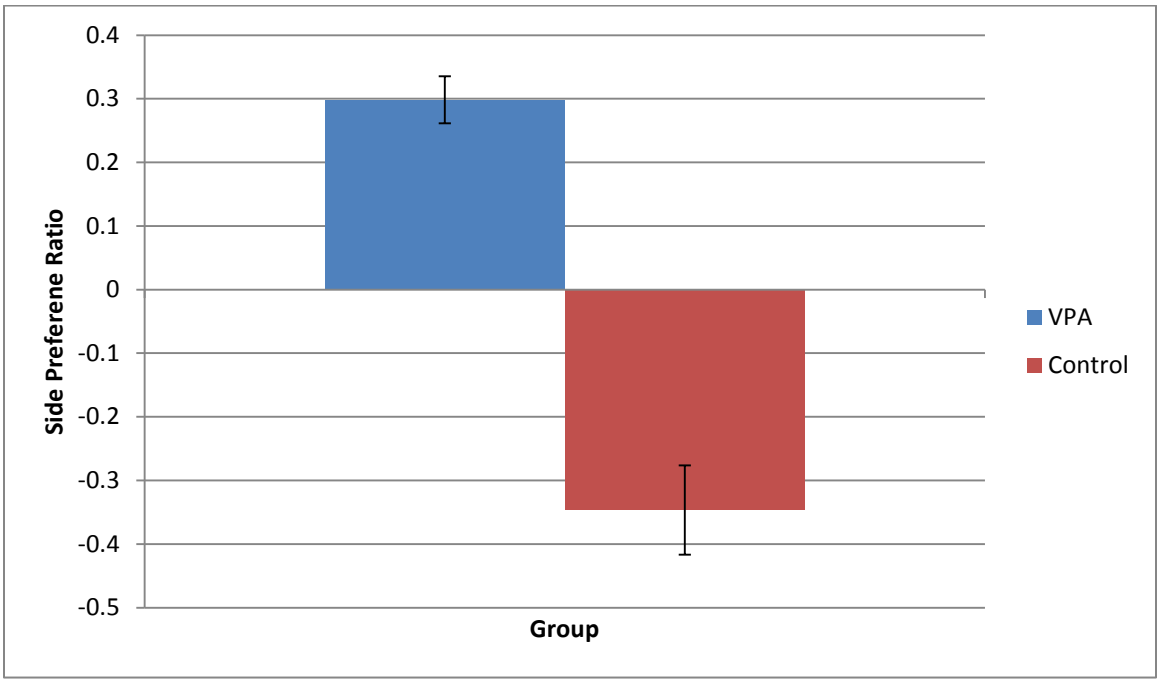


Figure 2: Graph showing object preference ratios for the VPA rats (n=9) and control rats (n=5). Error bars show standard error of the mean.

Escapes

Data were analyzed in a mixed-model analysis of variance (ANOVA). The main effect of day was significant, $F(4, 48)=3.781$, $p=0.009$, indicating a significant difference in number of escapes between the five days of testing. The main effect of group was also significant, $F(1, 12)=14.723$, $p=0.002$, indicating that the control rats exhibited a significantly higher number of escapes than the VPA rats. The interaction for group by day was not significant, $F(4, 48)=0.981$, $p>0.05$.

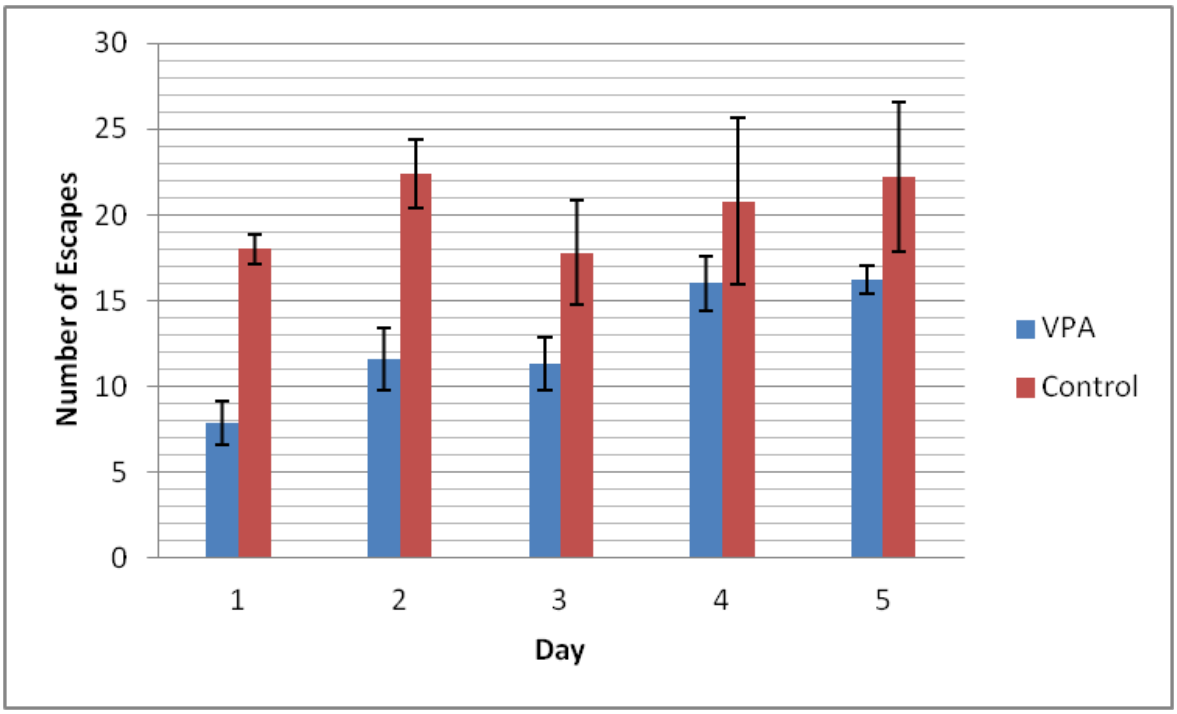


Figure 3: Graph showing average number of escapes each day for VPA rats (n=9) and control rats (n=5). Error bars show standard error of the mean.

Avoidances

Data were analyzed in a mixed-model ANOVA. The main effect of day was significant, $F(4, 48)=4.912$, $p=0.002$, indicating a significant difference in number of avoidances between the five days of testing. The main effect of group was also significant, $F(1, 12)=13.97$, $p=0.003$, indicating that the control rats exhibited a significantly higher number of avoidances than the VPA rats. The interaction for group by day showed a non-significant trend, $F(4, 48)=2.087$, $p<0.1$, suggesting that the number of avoidances for the control rats increased at a higher daily rate than for the VPA rats.

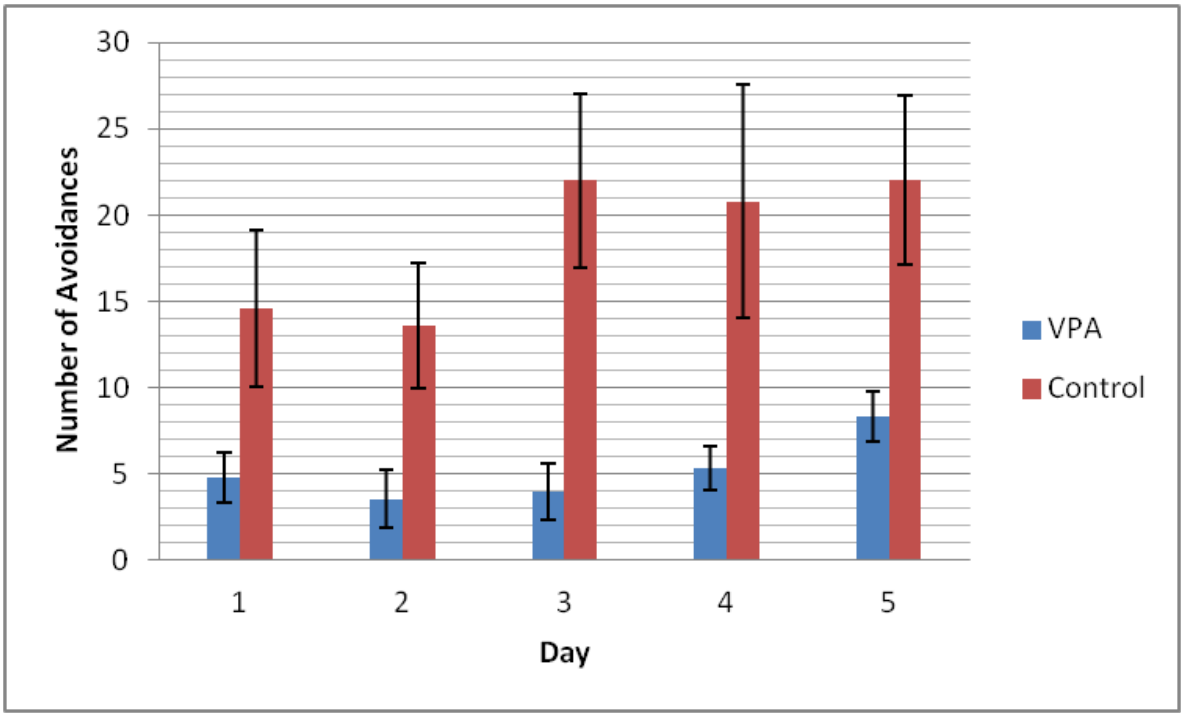


Figure 4: Graph showing the average number of avoidances each day for VPA rats (n=9) and control rats (n=5). Error bars show standard error of the mean.

Combined Escapes & Avoidances

Data were analyzed in a mixed-model ANOVA. The main effect of day was significant, $F(4, 48)=10.425$, $p<0.001$, indicating a significant difference in number of combined escapes and avoidances between the five days of testing. The main effect of group was also significant, $F(1, 12)=69.661$, $p<0.001$, indicating that the control rats exhibited a significantly higher number of combined escapes and avoidances than the VPA rats. The interaction for group by day was not significant, $F(4, 48)=0.519$, $p>0.05$.

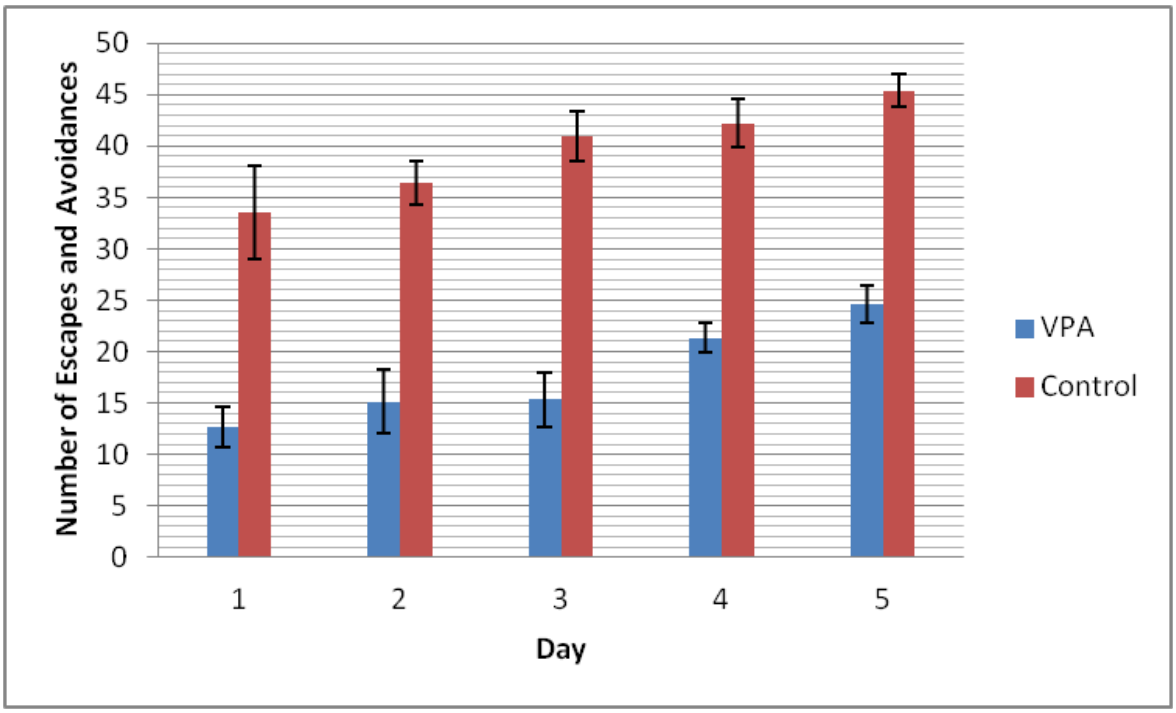


Figure 5: Graph showing the average number of combined escapes and avoidances each day for the VPA rats (n=9) and control rats (n=5). Error bars show standard error of the mean.

Discussion

An object preference test was completed in order to screen rats for autistic-like behaviors. Since restricted, repetitive, and stereotyped patterns of behavior, interests, and activities (American Psychiatric Association, 1994) is one of the core characteristics of autism, it was hypothesized that on an object preference test, the VPA rats would show a preference for a familiar object over a novel object, and in turn spend more time on the side of the testing chamber containing the familiar object. These hypotheses were supported, indicating a presence of behavioral symptoms of autism in the VPA rats. Since the symptoms of ASD are largely behavioral based, this helps to validate the VPA model of autism for use in the avoidance learning research.

Due to the co-association of ASD and learning disabilities, it was hypothesized that the VPA rats would learn to avoid the shock at a slower rate than the controls, and that in turn, the VPA rats would have a lower number of escapes and avoidances each day when compared to the control rats. The VPA rats did exhibit a lower number of both escapes and avoidances on each of the five days of testing, and a non-significant trend was found suggesting that the VPA rats did exhibit a slower rate of avoidance learning than the controls.

One clear observation in the data is that from day one the VPA rats exhibited significantly lower numbers of both escapes and avoidances than the control rats. As shown in figures 3 and 4, the VPA rats exhibited an average of 7.89 escapes and 4.78 avoidances on day one, while the controls exhibited an average of 18 and 14.6, respectively. This trend continued throughout the five days of testing. Other than differences in rate of learning, one possible

explanation for this is that VPA rats have been shown to have lower sensitivity to pain than controls (Markram et al., 2008; Schneider et al., 2008; Schneider, Labuz, & Przewlocki, 2001).

Limitations and Further Research

One major design flaw was ending the study after the fifth day of testing. When looking at the number of daily avoidances, the performance of the controls increased in the beginning and plateaued on day three, showing that the majority of the control animals had learned to avoid the shock by the third day of testing. However, the performance of the VPA rats actually declined on days two and three, and did not begin to increase until day four. If the study had continued for more than five days, it would have been possible to see how long it would take the performance of the VPA rats to plateau, and to see if their performance would ever reach the level of the controls. By doing so, it would be possible to tell whether the differences in performance between the two groups were actually due to differences in rate of learning, or if they could be attributed to something else.

Additionally, due to the differences in performance between the two groups, it would have been beneficial to test nociceptive reactivity in order to either rule out or provide evidence for differences in pain sensitivity as a possible variable. Past studies have used thermal paw withdrawal and tail flick tests to show that VPA rats have lower sensitivity to pain than controls (Markram et al., 2008; Schneider et al., 2008; Schneider, Labuz, & Przewlocki, 2001), but this has not yet been tested using an electrical stimulus. If differences in nociception were found, this would provide rationale for a future study testing avoidance learning using a shock of a higher amperage.

Furthermore, the small sample size could have impacted the results, as well as the fact that there were more rats in the VPA group (n=9) than in the control group (n=5). A future study with a larger sample size and equal representation in both groups could provide more accurate data by limiting the variability.

Conclusion

Although this study did have definite limitations, it also provided important insight in a new area of research, and a rationale for further research on avoidance learning using the VPA model of autism. Significant differences were found between VPA and control rats in terms of numbers of daily escapes and avoidances on a two-way active avoidance learning procedure, and a trend was found suggesting that the control rats learned to avoid at a faster rate than the VPA rats. By making some small changes, further research on the topic could examine whether these results are due to actual differences in rate of learning between VPA rats and controls, or whether they can be attributed to something else.

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