

The Effects of Dextroamphetamine on Conditioned Place Preference and Locomotion in a
Valproic Acid Rat Model of Autism



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Patricia Todd

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The Effects of Dextroamphetamine on Conditioned Place Preference and Locomotion in a
Valproic Acid Rat Model of Autism

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Presented By:

Patricia Todd

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Pledge: ___ Patricia Todd ___

Approved By:

_____ Dr. Jeff Hollerman _____

[First Reader]

_____ Dr. Rodney Clark _____

[Second Reader]

Abstract

Autism Spectrum Disorder (ASD) is a disorder that is prevalent in today's society, yet the exact etiology is unknown. It is thought to be a matter of genetic and environmental factors that are involved in ASD. Valproic Acid (VPA) intake by humans has been found to be a significant risk factor for ASD, and a model in rats was created and validated using this knowledge. It is theorized that a malfunction or dysfunction in the dopamine system may be a part of the etiology of autism. The dopamine system is strongly associated with reward, and can be stimulated using amphetamines. The dopamine system has also been closely associated with locomotion, with higher levels of dopamine function causing hyperlocomotion. The present study aimed to analyze whether d-amphetamine had a reduced effect in the VPA model in relation to the control, in terms of conditioning and locomotion. In the present study, two groups of male rats were used consisting of a group of VPA model rats and a control group (n=6/group, n=12 total). The rats were conditioned with d-amphetamine using a conditioned place preference paradigm. Additionally, rats' locomotion was tested based on beam breaks along an X,Y infrared beam axis, with Z axis motion counted manually. The conditioned place preference test did not yield significant results between groups with controls showing similar levels of conditioned preference. The locomotion test yielded the result of a significant difference between groups in Z-axis motion, with VPA rats showing a smaller increase to amphetamine than controls; however, no significant difference in amphetamine effects between groups for X,Y axis movement was found.

Key Words: Autism, Valproic Acid, d-amphetamine, Dopamine, Conditioned Place Preference, Vertical and Horizontal Locomotion, Rat

Introduction

Autism Spectrum Disorder, otherwise known as ASD, is a heterogeneous group of disorders that vary in symptom pattern and level of function. It is characterized by communication deficits, social impairment, and repetitive behaviors (American Psychiatry Association, 2013). In terms of prevalence, 1 in 68 individuals in the United States and Canada are diagnosed with ASD by the age of 8 (Baio, 2014). There is currently no directly known cause of ASD; it is theorized to be a combination of inherited genetic risk factors and in utero environmental risk factors that result in ASD in a human. These environmental risk factors may induce biological changes in the fetus, or in the mother themselves prior to conception resulting in changes in inherited genetics. In terms of genetic factors, it is theorized that 400-1000 genes may be risk factors leading to the development of ASD (Devlin and Scherer, 2012). Environmental risk factors include *in utero* exposure to toxins, pesticides, and drugs, along with infections (Harden, 2013).

Valproic Acid as a Risk Factor for Autism:

In utero drug exposure has been shown to cause autism or autism-like phenotypes. More specifically, valproic acid (VPA) exposure *in utero* is known to result in a higher incidence of autism (Romoli et al., 2019). VPA is used as an anti-epileptic drug (AED) in humans, and is one of the most prescribed AEDs worldwide (Romoli et al., 2019). When taken orally, the drug functions to increase neurotransmitter release of γ aminobutyric acid (GABA) and transmission in the brain (Löscher, 1993). GABA functions as a neurotransmitter, binding to GABA receptors and inhibiting neuronal activity. Additionally, VPA blocks excitatory postsynaptic potentials of neuronal excitation that are coming from N-methyl-D-aspartate (NMDA) receptors (Romoli et

al., 2019). The decrease in excitation allows for the threshold for seizure activity to be reached less often, which reduces anxiety, stress, and seizure activity for the patient (Romoli et al., 2019). A seizure occurs when there is a high amount of discharge of neurons in the brain, and the brain's normal electrical signals are disrupted (Stafstrom and Carmant, 2015). The heightening of the threshold for seizure activity, by VPA, allows the brain to be able to better control the signals when a seizure occurs, making VPA an effective means of seizure control for some people.

VPA exposure in utero:

Different clinical studies have been conducted observing the effects of intake of VPA during pregnancy. One study found that VPA exposure during the first trimester of pregnancy caused a higher incidence of neural tube defects, along with other congenital malformations (Jentink, 2010). Additionally, VPA administration during the first trimester has been associated with an increased risk of impaired cognitive function, i.e. lower iq scores in comparison to the mother, along with being a risk factor for autism (Meador et al. 2009). There is a higher incidence of autistic-like symptoms in children with fetal valproate syndrome, which has been confirmed by multiple studies. One study found that *in utero* exposure of VPA had an absolute risk of 4.42% of ASD in offspring, in reference to people who didn't take valproic acid who had an absolute risk of 1.52% of ASD in offspring (Christensen et. al., 2013). Another study found that there was a 10-fold increase in neurodevelopmental disorders in children exposed to VPA polytherapy and a 6-fold increase in children exposed to VPA monotherapy, with Autism Spectrum Disorder being the most prevalent (Bromley et al., 2013). Despite all of the research conducted, there is no definable reasoning as to why it causes ASD or its phenotypes.

VPA in a Rodent Model:

Valproic acid is a known factor that creates a higher incidence of autism in humans. When VPA is administered *in utero* to rats, it creates autism-like phenotypes with symptoms that are associated with ASD. These phenotypes include decreased social interactions, lower exploratory activity, communication deficits, along with hypolocomotion and repetitive/stereotypic-like activities (Schneider and Przewlocki, 2005). These symptoms are reflected in humans who are diagnosed with ASD, validating VPA's efficacy in the creation of an ASD model in rodents in terms of behavior alterations. Additional studies have also shown the similarities in cellular and molecular alterations in addition to behavioral alterations in order to prove the validity of the VPA model for ASD (Chomiak et al., 2013).

Autism-Linked Deficits of VPA vs ASD:

Sex Differences:

In terms of the behavioral symptoms of ASD, there is a difference in sexes in both the rodent VPA model for ASD and in humans with ASD. In humans, there is a between a 4-5:1 ratio of males to females diagnosed with ASD (Baio, 2014). However, in the VPA model, rats are affected at a 1:1 male to female ratio in terms of behavioral deficits and ASD-like mannerisms. Looking closer at sex-specific effects, VPA-exposed female rats are known to perform better than VPA rat males on social interaction sniff tests meaning that they do not seem to have the same level of social deficits as male rodents have (Katoaka et al., 2013). A social interaction sniff test is when two rodents of the same gender are placed in an area together, and the amount of anogenital and facial sniffs that occur within a set period of time, is recorded. The more the rats sniff each other, the more they are considered to communicate and interact through

sniffing. In the study by Katoaka et al. (2013), scientists found abnormal performance in the sniff test for males, while finding relatively normal performance by the females in relation to the control rats. These test differences showed that male rats are more affected in terms of this social behavior deficit than females are in the VPA rat model. This disparity may be paralleled in humans, as females are known to be less likely to receive a diagnosis of ASD due to the fact that they often perform similarly as a neurotypical person in social situations despite possible deficits. Male humans, however, have a tendency to perform worse in social situations in terms of communication and other deficits and therefore are more readily diagnosed under the umbrella of ASD. This difference between females and males further supports the validity of the VPA model in terms of social interaction and its relation to humans. Additionally, this is one reason why males will be used in this experiment, as they better reflect today's understanding and diagnostic testing of the ASD -model. Using male rodents for this experiment will allow for confirmation of ASD phenotypes being present, as the social inhibition should be more easily observable. In using female rodents, it would be more difficult to observe that the VPA treatments were effective in creating the desired model with social tests.

Why the Deficits?:

Social behavior and interaction defects are a large part of autism, especially in males, as the deficits associated with it affect everyday life for those with ASD; however, the cause of this deficit is currently unknown. One motivation for engaging in social interaction would be the reward signals received during said interaction. If a person isn't receiving those reward signals and interpreting the stimulus on hand, i.e., social interaction, as positive, then they may not seek out future social interactions. In terms of not receiving reward signals, previous studies suggest that different neurotransmitter systems, such as the dopaminergic (DA) system, are part of the

etiology of ASD; however, the neurotransmitter systems and their changes are currently unknown (Nguyen et al., 2014). The DA system is known as the “reward system” and is involved in the consolidation of memory, including drug-seeking and reward incentive learning (Arias-Carrión et al., 2010). Additionally, it is suggested that the DA system is involved in behavioral choices based on rewards (Arias-Carrión et al., 2010).

The Dopaminergic System:

In terms of how the DA system functions, a positive stimulus is introduced, and it is interpreted as rewarding. Receiving a “reward” sends a signal to the brain that causes a dopamine release in response to the stimulus. The dopamine is released into the synaptic cleft so that it can interact with the postsynaptic neuron. The dopamine then binds to dopamine receptors, creating a signal that is relayed through the mesocortical pathway. The brain then continues to send these signals which stimulates the reward and pleasure centers of the brain. Having associated the stimulus with feelings of pleasure creates a drive to seek out the positive stimulus in the subject. When a certain stimulus doesn’t have a rewarding effect, the cause could be that it isn’t stimulating the DA system. In this case, the subject will not be motivated towards the stimulus in the future. There may be a reduction in release of dopamine or in the response by the postsynaptic neuron, which means that the stimulus may only be able to activate the brain at higher levels of neurotransmitter release than a neurotypical organism’s levels of activation.

The DA system can be altered or become dysfunctional in a number of ways. When a gene is changed, the system cannot act as normal. Different studies have found mutations of different genes within the DA system, including DA transporters 3, DA receptors, and enzymes of DA synthesis to possibly be a part of the etiology of ASD (Nguyen et al., 2014). However, no

exact one mutation has been shown to cause ASD. When the DA system is functioning at a deficit, the brain may not be signaling reward normally and therefore the subject is not learning or is learning differently than the average subject. In one study, dopamine transporter knockout mice were created, and it was found that they appear to be less sensitive to rewarding stimuli than the wild-type (Cinque et al., 2018). In this study, a test was also conducted in which reward incentivized behavior and learning was tested, and the KO DAT mice were found to perform less of the behavior for the reward (Cinque et al., 2018). This study reflected the idea that the DA system, and its function, is very important in the process of learning and behavior reinforcement. The DA system and the brain's learning from incentives may be reasoning as to why ASD diagnosed humans are not motivated to communicate socially or to engage in social interaction. It is currently theorized in the scientific community that the dopamine system may play a part in the phenotypes of autism in humans; however, there is not a lot of research yet in terms of dopamine dysfunction in humans. One possible theory is that if they are not receiving a "reward" and/or dopamine release from social interaction, then there is no incentive to continue it in the future. This lack of incentive may create the phenotype of decreased social interaction and communication.

DA-induced Hyperlocomotion:

The VPA rat model, as previously stated, is known for having hyperlocomotion as one of the autistic-like phenotypes it has. This connection relates to the dopamine system in the fact that dopamine neurons are known to modulate locomotion in vertebrates, as well as reward processes (Ryczko and Dubuc, 2017). Dopamine functions as a neurotransmitter to send activation signals to the brain stem locomotor networks via the nigrostriatal pathway (Ryczko and Dubuc, 2017). When there is an increase in dopamine neurotransmitter activity in the brain,

one effect of this is increased locomotion. One particular study focused on dosing VPA model rats with METH-amphetamine and recorded the locomotion before and after dosage with METH (Hara et al., 2015). This study found that VPA rats had lower METH-induced hyperactivity than the control groups in terms of males (Hara et al., 2015). This means that while the male rats still had a reaction that increased locomotion, the stimulant did not have as great of an effect in the VPA rats as it did the control group. The dopamine system has been shown to be affected in some way to have caused this difference between groups, meaning that some part of it may be altered or have become dysfunctional in the average VPA model rat.

Dextroamphetamine Administration:

In terms of male VPA-exposed models and their reward systems (their DA systems), a way to test reactivity is through the administration of amphetamines. Amphetamines are drugs that interact with monoamine uptake, via translocation of monoamines as the amphetamine first begins to enter the cell. The increased amphetamine concentration within the cell causes even more amphetamine molecules to influx, while competing with monoamines. This influx of amphetamine causes a displacement of monoamines within the neurons, and eventually causes the reuptake transporters to reverse. Neurotransmitters are then pumped back out of the neurons and into the synaptic cleft. Additionally, amphetamines function to block dopamine and norepinephrine terminals for reuptake. This increases the amount of norepinephrine and dopamine in the synaptic cleft, in the case of d-amphetamine. These neurotransmitters left in the presynaptic cleft then continue to cause signals in the postsynaptic neuron (Heal et al., 2013).

More specifically to d-amphetamine, it increases DA's efficacy and enhances midbrain DA function (Arias-Carrión et al., 2010). At high levels, amphetamines also may inhibit DAT,

which results in a large dopamine increase in the synaptic cleft as well (Quevedo 2021). This lack of reuptake causes there to be more reward signals sent, as the brain is positively stimulated from the greater amounts of DA in the synaptic cleft. In a control subject, the subject would seek out the amphetamine associated stimulus rather than a neutral stimulus due to the fact that it is seen as a positive stimulus. This is known drug-reward seeking behavior. Also, the subject typically experiences hyperlocomotion, as the greater dopamine release causes greater signals to be sent to the brainstem locomotor networks (Ryczko and Dubuc, 2017). However, it is suggested that the dopaminergic system functions differently in the ASD model. The fact that it might function differently, as previously discussed, means that there may be different cues and reinforcement that someone with ASD is receiving in comparison to the signals that the average person is getting. By testing a subject's reaction behaviorally to amphetamines, it is possible to test whether the subject still receives the same signals neurally due to the positive stimulus as the average person does, or if their DA system is not sending those signals in the same way for some reason.

In terms of tests that can be performed with amphetamines in the VPA model, one particular study researched the reactivity to N-methylamphetamine (METH) in VPA rats, and found that there was a reduced increase in DA release in comparison to the control, in addition to reduced METH- induced hyperactivity in the VPA model for males, but not in VPA model females (Hara et al., 2015). These findings suggest that the DA system of the VPA rat does not function at as high of a rate as it does in the control rat. Additionally, the researchers found a reduced increase in c-fos expression in the prefrontal cortex in male rats, which is associated with DA1 and DA2 receptors along with other dopaminergic receptors functioning in the prefrontal cortex (Hara et al., 2015). This increase in c-fos expressions means that there is a

reduced increase in the DA system's activity in response to amphetamines. This study, by Hara et al. in 2015, suggested that the dopaminergic system could be responsible for these alterations in behavior and hyperactivity that are associated with the VPA model, and therefore the ASD model. The dopaminergic system is suggested to be part of the etiology of ASD, and this is reflected in VPA rat models as well.

Amphetamine in a Conditioned Place Preference Paradigm:

The conditioned place preference paradigm (CPP) is a commonly used model for studying reward-related behavior in relation to a positive or negative stimulus (ie. drug). The apparatus used consists of three chambers, one of which has no stimuli to function as a neutral chamber while the other two have discriminatory stimuli via visual or somatosensory cues. Initial preference of the subject is measured between the two discriminable chambers by allowing them to roam freely between all three chambers for a given period of time. The initially non-preferred chamber is paired with the drug treatment if it is a positive stimulus, or the initially preferred chamber if it is a negative stimulus. By performing these pairings during treatment, it creates an association between the environment and the treatment in order to test if the treatment is strong enough in reward or aversive effects to change the initial preference of the subject. The pairing of the drug with one chamber and the pairing of the drug vehicle with the opposite chamber takes place on alternating sessions, where the subject is given the treatment and then placed in the chamber for the given period of time. After these sessions are performed, CPP can be measured by using the same test for initial preference of allowing the subject to freely roam the three chambers for a given period of time. The pre-test and post-test CPP scores can be calculated for comparison, which can allow experimenters to know if the drug was effective in changing preference (Prus et al., 2009).

Many studies have found that amphetamines function to effectively create a CPP using doses of amphetamine paired with the CPP paradigm (Grinberg and Einat, 2011, Prus et al., 2009). This pairing can be related to the previously mentioned fact that amphetamine effectively stimulates the reward pathway. By stimulating it, it creates a conditioned place preference towards the environment that is paired with the amphetamine. This is what makes the CPP paradigm an effective model to use to test dopamine response from amphetamine dosages. In this study, drug-reward seeking behavior of a VPA rat will be tested, using d-amphetamine, in comparison with a control rat, in order to analyze the dopaminergic system's effects on locomotive behavior and conditioned place preference.

Methods

Prenatal VPA Exposure:

Female Sprague-Dawley rats were bred with male, Sprague-Dawley rats. The bedding was checked multiple times a day for a sperm plug, and then the male and female were separated when it was found. The day the sperm plug was found was considered gestational day 0. Female rats received 800 mg/kg of sodium valproate mixed with peanut butter on gestational day 12.5. Offspring were housed with dams and separated at postnatal day 21 by sex. Male VPA model subjects and male control subjects were used for this study and housed with littermates in groups of 3. Food and fresh water were provided ab libitum.

Animals:

Male Sprague-Dawley rats were the subjects of the study at week 8 of age, which is the age of adulthood. They were housed and maintained under a 15:9-hour light-dark cycle at a constant temperature of 23°C. Food and fresh water were provided ab libitum. Rats were handled

for 3-5 minutes each for 4 days before experimentation. The experimental groups consisted of a control group and a VPA model group, each with six rodents.

Drugs:

D-Amphetamine was obtained from Sigma Aldrich. It was administered at a volume of 1 mg/kg to each animal (Galen et al., 2019). It was injected via intraperitoneal injections using a 12 mm, 26-gauge needle.

Conditioned place preference protocol:

A 3-chamber apparatus was used, which was a painted wooden box with 3 compartments. The box had measurements of 39x13x16 inches. Each of the compartments were 8x13x16 inches. The two outer chambers were fitted with rubber mats for discrimination, a ribbed mat in the chamber with black and white checkered painting on the walls and a smooth rubber mat in the fully black painted chamber. The chamber in the center was a neutral space painted white with wood as the only flooring. A stopwatch was used to record the amount of time spent in each of the three chambers. This protocol was based on Sun et al., 2018.

Locomotion Testing:

Following the CPP testing, each rat was placed into the Locomotor Activity Monitor apparatus (Columbus Instruments, Columbus, OH). This piece of machinery compiled data based on how much the rat moved throughout the box, passing through X,Y coordinates of infrared beams. They were placed in the apparatus and tested for a duration of 15 minutes directly following the CPP conditioning. The number of times they crossed the X,Y coordinates were recorded for measurements of locomotion as beam breaks. The amount of times they

reared up on their hind legs or stood on their hind legs was recorded for the purpose of z axis data via visual inspection.

Pre-condition:

Each rat was preconditioned for one day. The rats were placed in the center (neutral) chamber of the 3-chamber apparatus for 15 minutes. They were allowed to freely roam the three chambers during this time. The rat's initial preference was recorded based on the amount of time spent in each chamber. Entrance of each chamber was defined as when the front two paws crossed the chamber door frame.

Conditioning:

Over the course of 6 days, subjects were treated with d-amphetamine (dosage) on alternating days and given no treatment on opposite days. No treatment was given when assigned to their initially preferred chamber, and they were not able to switch chambers as the chamber was closed off from the other chambers. When given the stimulant of d-amphetamine, the rats were assigned to the initially non-preferred chamber. They spent 30 minutes in the respective chamber during conditioning. The rats' place preference was tested 24 hours after the final dosage. Each rat was placed in the center, neutral chamber and allowed to explore freely for the test for duration (15 minutes). The amount of time spent in each chamber was recorded for analysis of place preference.

Results

X,Y Locomotion:

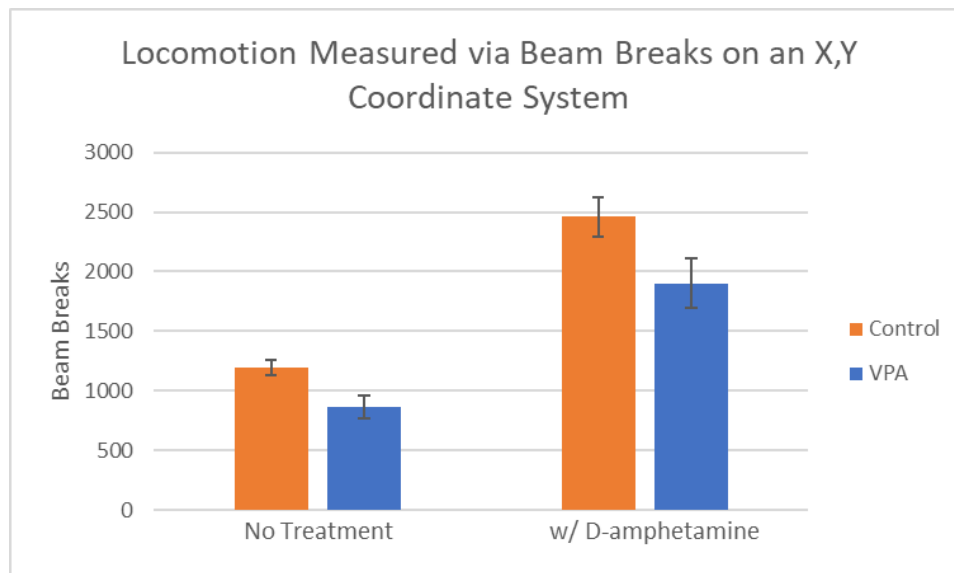


Figure 1: Locomotion was recorded in an X,Y coordinate system as beam breaks with and without treatment with 1.0mg/kg, i.p. d-amphetamine. The difference between VPA and Control was found to have a significance of $p < 0.05$, and the difference between treatments was found to be significant ($P < 0.05$). A significant effect was not found in the interaction within groups and treatment. Error bars represent $\pm$ SEM.

A Two-Way Mixed ANOVA was run on the locomotion data, relative to X,Y coordinate beam breaks. This data shows a significant difference within treatments, meaning that X,Y beam breaks were higher for both groups following treatment than with no treatment ($F=71.136$, $df=1$, $p < 0.001$). The test showed a significant difference between subjects, meaning that X,Y beam breaks were lower for the VPA group in relation to the control group with no treatment ($F=8.758$, $df=1$, $p=0.014$). From this data, it can be noted X,Y beam breaks were also lower for the VPA group in relation to the control group with d-amphetamine dosage. However, there is not a

significant difference in the interactions between the treatment and the group ($F=0.684$, $df=1$, $p=0.428$). This means that both groups were affected by the d-amphetamine at similar levels.

X,Y beam breaks increased at the same rate following treatment, in relation to without treatment for both groups. There was no main effect across treatment within groups, which can be seen in Fig. 1.

Z- Locomotion:

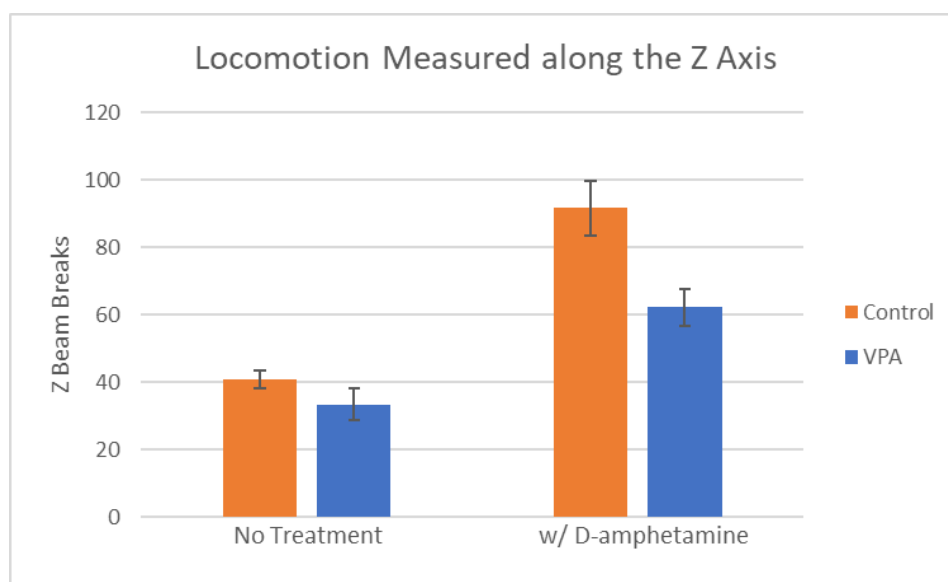


Figure 2: Locomotion was recorded along a Z axis with and without treatment with 1.0mg/kg, i.p. d-amphetamine. The difference between VPA and Control was found to have a significance of $p<0.05$, and the difference between no treatment and treatment with d-amphetamine was found to be significant ($P<0.05$). The difference within treatment and group interactions was also found to be significant with $p<0.05$. Error bars represent $\pm$ SEM.

To analyze locomotion data along the Z-axis, a Two-Way Mixed ANOVA was used. The results yielded a significant difference between groups in that the control group had higher levels of Z-axis motion than the VPA model without treatment ($F=8.344$, $df=1$, $p=0.016$). The control

group can also be noted to have higher levels of Z-axis motion than the VPA model with treatment also from these results. The significance in the data within treatments shows that both groups had increased Z-axis motion following dosage with d-amphetamine in relation to their base levels of motion with no treatment ($F=75.495$, $df=1$, $p<0.001$).

Significance between groups within treatments yielded a significant difference ($F=5.794$, $df=1$, $p=0.037$). To further investigate this significance, a post-hoc test was then run with Bonferroni adjustment to test the difference between groups within treatments. The test revealed that all comparisons between groups had a $p_{\text{bonf}}<0.05$ except for the comparison of control with no treatment and VPA with no treatment ($SE=7.866$, $M=7.444$, $p_{\text{bonf}}=1$). The comparison between control with d-amphetamine treatment and VPA with d-amphetamine treatment was significant ($SE=7.866$, $M=29.5$, $p_{\text{bonf}}=0.009$). Control with no treatment and control with d-amphetamine treatment were statistically significant ($SE=6.479$, $M=-50.833$, $p_{\text{bonf}}=<0.001$). The difference between VPA with no treatment and VPA following d-amphetamine dosage was significant ($SE=6.479$, $M=-28.778$, $p_{\text{bonf}}=0.008$). The control model had a greater, positive difference in locomotion with versus without treatment, than the VPA model. The difference between groups (approximately 22.2045), within treatments, suggests that the control model experienced greater levels of d-amphetamine-induced hyperlocomotion than the VPA model. The interaction of d-amphetamine had reduced effect in the increase of locomotor activity in the VPA model in relation to the Control model, which can be noted in Fig. 2.

Conditioned Place Preference:

Conditioned Place Preference (CPP) scores were calculated via a ratio of time spent in the non-preferred chamber to the time spent in both stimulus-paired chambers. A change in CPP score signifies a change in preference, such as a lower score means less time was spent in the non-preferred chamber. These scores were calculated for both the pre-test data and the post-test data.

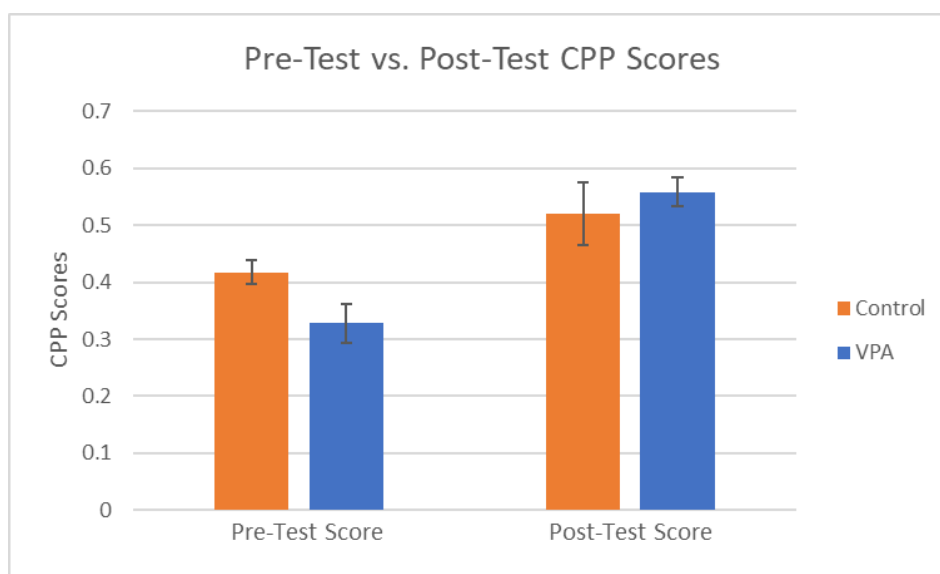


Figure 3. Pre-test and post-test CPP scores were recorded and compared by group in order to test conditioned place preference changes following conditioning. A significant difference can be seen between pretest and post-test scores ($p < 0.05$), showing that significant place preference to amphetamine developed. No significant difference can be noted between groups and there was no interaction ($p > 0.05$). Error bars represent $\pm$ SEM.

A Two-Way Mixed ANOVA was used to test pre-test versus post-test conditioned place preference scores. A significant difference was found between CPP Scores independent of groups, meaning that all groups experienced positive conditioning towards the conditioned

chamber. ($F=18.397$, $df=1$, $p=0.002$). No significant difference was found in the interaction between treatments and groups, meaning that there was no difference in conditioning between the VPA group and the control group in terms of the treatment's effect on conditioning ($F=2.627$, $df=1$, $p=0.478$). Additionally, the test showed that there was not a significant difference between the VPA and the control group's pretest CPP scores or the post-test CPP scores ($F=0.544$, $df=1$, $p=0.478$). No main effect can be noted in relation to treatment within groups, as can be seen in Fig. 3.

Discussion

This study explored the effects of d-amphetamine in relation to conditioning and how it affected locomotion. Locomotion was observed in the form of ambulatory motion and exploratory motion. D-amphetamine was used to condition rats using a 3-chamber place preference test and conditioning them towards the least preferred chamber with doses of the d-amphetamine. It was hypothesized that d-amphetamine would be a positive stimulus for the rodents, but that the VPA rats would have a reduced shift in place preference towards the initially non-preferred chamber in relation to the control rats. The animals were then tested, following with and without dosage of d-amphetamine, in a Locomotor Activity Monitor apparatus to test for changes in ambulatory and exploratory behavior based upon x, y, and z beam breaks. It was hypothesized that the d-amphetamine would have a reduced stimulatory effect in the VPA model versus the control model in relation to locomotion.

Conditioned Place Preference:

The conditioned place preference test resulted in a significant difference between pre- and post-test scores; however, there was not a significant difference between VPA and control or

any interaction (Fig. 3). This lack of an interaction suggests that while the conditioning with d-amphetamine was effective in increasing CPP scores for both groups, the change in the VPA group was not significantly different from that of the control group (Fig. 3). This does not support the hypothesis that the VPA model would produce reduced conditioned place preference.

These results may have occurred due to the method of giving a dosage of d-amphetamine via injection, in the absence of a saline injection on alternate days, followed by immediate placement in the apparatus during conditioning. The painful stimulus of the shot may have affected the control's CPP score more than it affected the VPA group's CPP score, due to the fact that the VPA model is theorized to have a higher threshold to nociceptive pain. One study restrained VPA rat subjects by hand and directed radiant heat onto their tails while recording tail flick measurements and paw withdrawal (Schneider and Przewlocki, 2013). They found that VPA rats had a significantly higher threshold to nociceptive pain in relation to a control subject (Schneider and Przewlocki, 2013). This difference in pain tolerance and lack of saline injection may have contributed to the lack of significant results in the CPP scores.

The lack of statistical significance in the results of the CPP testing also may have occurred due to small sample sizes. Sample sizes being too small can lead to more of a chance of drawing a false-negative or false-positive conclusion (Noordzij et al., 2011). Having a group size of 6 rats may have increased the chance of outliers affecting data or significance being lost from the experiment and the trials. A larger group size may have yielded a more significant trend or results and would be a good potential start for future research on this hypothesis.

XY Locomotion:

The locomotion testing resulted in a significant increase in locomotion for both groups following d-amphetamine administration. (Fig. 2). A significant difference was observed between groups in terms of locomotion, meaning that their baselines of locomotion were different (Fig. 2). However, d-amphetamine administration resulted in a non-significant difference in the increase in locomotion between VPA and control (Fig. 2). This lack of significance implies that d-amphetamine affects the VPA model and the control to the same extent for locomotion. This data does not support the hypothesis that there would be a reduced increase in locomotion found in VPA in relation to the control model's increase in locomotion.

In relation to supporting the data, one study looked at the VPA model in relation to locomotive response to a meth-amphetamine dosage of 0.5 mg/kg and administered it prior to an open field test that tracked horizontal motion (Schiavi et al., 2019). They found significance before and after treatment for both the VPA group and the control group; however, they also did not find statistical significance in terms of a difference between the groups due to administration (Schiavi et al., 2019). These results reflect the fact that there may not be differences in the VPA model for autism in relation to horizontal locomotion, in response to amphetamines (Schiavi et al., 2019).

In support of the hypothesis, the study by Hara et al., previously mentioned in the introduction, did find that there was a reduced METH-induced hyperlocomotion in the VPA model in relation to the control model (2015). This study injected the subjects with Methamphetamine hydrochloride via IP injection at a dosage of 10 mL/kg (Hara et al., 2015). They used a locomotor apparatus which was a transparent acrylic box, with a black floor and

were allowed to habituate before being injected with the METH, and then their horizontal locomotion was measured via a-y coordinate sequences using a Panlab Infrared Actimeter System (Hara et al., 2015). The study found a significant difference between the male control group and the male VPA group, showing a reduced increase in locomotion resulting from the METH dosage (Hara et al., 2015). The method was similar to the method in the present study, suggesting that significance in the present study should have been found between groups. However, significance was not found. This may have been due to the difference in dosage or in the method of having a habituation period prior to testing not occurring in the present study. It is also possible that using d-amphetamine versus using methamphetamine may have caused a difference in response in this study. Low group sizes may have also contributed to the lack of statistical significance.

Z Locomotion:

Statistical significance ($p < 0.05$) was found in Z axis locomotion within groups, between groups, and between treatments (Fig. 1). VPA was found to have a reduced increase in z-axis movement following d-amphetamine administration in relation to the control model's increase in z-axis movement following treatment. This statistical significance supports the hypothesis that d-amphetamine has a reduced effect in locomotion in the VPA model in relation to the Control model.

Amphetamine exposure in a regular rat model has been found to significantly increase rearing activity associated with increases in dopamine (Mihalcikova et al., 2019). The result of amphetamine increasing locomotion along the Z-axis was expected. However, lower exploratory activity has been noted in the VPA model in the form of rearing when measured in a small open

field test (Schneider and Przewlocki, 2013). This initial difference in locomotion is reflected in the baseline measurements of locomotion without treatment, reflecting validity of the VPA model of ASD. Following treatment, the significant difference between groups supports the hypothesis of an increase in locomotion while also supporting the idea that d-amphetamine affects the exploratory behavior of these models in different ways.

The lack of statistical significance in the X,Y Locomotion while Z locomotion had significance may have been due to the fact that an animal cannot be moving along the X,Y axis while it is rearing up in the Z axis. This may have been a confounding variable across the data sets, and increasing time measured or limiting X,Y versus z motion to different tests may have fixed this limitation. Small sample groups may have also caused this lack of significance.

Future Research:

The results of this study yield many possible areas of future research for those interested. More research could be performed on the discrimination between vertical and horizontal locomotion. Alternatively, this experiment could be performed with a larger sample size to see if significance still holds true. If this research study were to be replicated or repeated, amendments to the procedure would be recommended. Using a camera or a sensor system for z-axis locomotion would be recommended, as manual counting is prone to error and inconsistent at times. Additionally, adding a time period for acclimation from the injection prior to being placed in the conditioning apparatus may prevent any negative associations between the pain and the chamber. In relation to chambers being associated with pain, injecting the rats with saline when being placed in the initially preferred chamber may prevent any pain discrimination from occurring between chambers in relation to CPP scores.

D-amphetamine, in this study, was not found to have significantly different influences on conditioned place preference and had few different influences on locomotion in VPA as opposed to control rats. D-amphetamine did yield an effect on vertical axis motion, suggesting it has a reduced effect in exploratory behavior in the VPA model. This study yielded results that could be beneficial for future researchers in the field of autism and rodent studies. If the etiology of autism, or pieces of it are further researched then it could yield great advances in medicine. The dopamine system is believed to be involved in the etiology of the symptoms and phenotypes of autism, and variations on this research may yield clearer results in support of this hypothesis.

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