A Conformational Study for the Validity of a Valproic Acid Induced Rat Model of the Autism Spectrum Disorder

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Introduction

Autism Spectrum Disorder (ASD) is a group of developmental disorders best known for the debilitating social impairments that prevent ASD patients from interacting and communicating with others effectively. The spectrum refers to the vast array of symptoms which characterize ASD including behavioral, communication, and learning difficulties. The work presented here seeks to confirm the validity of a drug induced rat model of ASD. Although it is known that there is a strong genetic correlation to developing the disorder previous studies have also shown that not very most of the ASD related genes fail to produce the disorder alone. Patients exposed to certain environmental factors in conjunction with the genetic element suffer the highest risk of developing ASD. Prenatal exposure to Valproic Acid (VPA) has been isolated as one such environmental influence. Fortunately previous studies have shown inclinations that the same kind of prenatal exposure to VPA in rats can lead to a reliable animal model of ASD, with many of the same social and behavioral complications seen in human patients. Our studies used pregnant female rats exposed to VPA roughly around the period when the cranial neuronal tube begins to close in order to confirm the validity of this ASD animal model. Briefly, after the pups were born a series of behavioral studies, such as ultra sonic vocalization analysis and open field tests, were conducted to determine whether this rat model indeed exhibited a similar symptomology to that of human ASD patients. Previous studies show that this rat model causes long term social deficits. This study corroborates with the literature by showing increased anxiety levels and aimless repetitive behaviors known as stereotypies in the VPA treated animals.

Methods

Pregnant adult rats received IP injections of VPA on gestational day 12.5 at 600 mg/kg doses. After the pups were born they were weighed and examined for deformities every five days until post natal day (PND) 20.

Ultra Sonic Vocalization: The rat pups are first isolated from the mother for a period of 25 minutes prior to recording. The pups are then placed one-by-one into a Styrofoam box, which helps prevent echoes, and recorded using the Seawave Pro Version 2.0 spectrograph recording program for 2 minutes each. Recordings were taken for each rat pup every 5 PNDs starting with PND 5 to PND 20. Each recording was analyzed and every vocalization was classified for type of call from a list of call types exhibited in Figure 2.

Open Field Tests: Each rat was placed in separate animal activity chambers (40x25x20 cm) acquired from AccuScan Instruments, which feature a 16 box laser grid and recorded for a total of 5 minutes. These chambers use a Digipro program to analyze the break patterns of the beams and categorize the subject's movements as in rest, locomotion, or stereotyped behavior.

Data Analysis: All statistical data analysis was preformed using the Graphpad Prism 6 software.

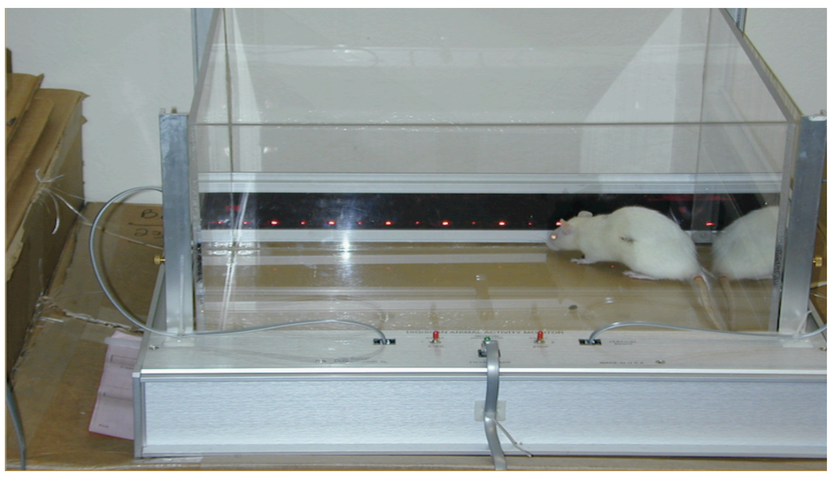

Figure 1: The animal activity chamber from Accuscan Instruments

Results

After analysis of the open field test the data shows that the VPA treated animals traversed significantly less of the field within the allotted 5 minute period (See Figure 2). Compared to the control group, the VPA subjects traversed the margin or perimeter of the field less, but not enough to be significant (See Figure 2). It also shows that VPA subjects traversed the center of the field, that is they interrupted beams anywhere other then the margin of the grid, significantly less than the control saline treated rats did (See Figure 2). Figure 3 represents data from the Ultra Sonic Vocalizations tests and it reveals an inverse trend in the total number of vocalizations between the two groups. As the study progressed the VPA group had less and less vocalizations whereas the control group had a steady increase in vocalizations from PND5 to PND20 (See Figure 3). Figure 3 also shows that by PND 20 the two groups had about the same total number of vocalizations.

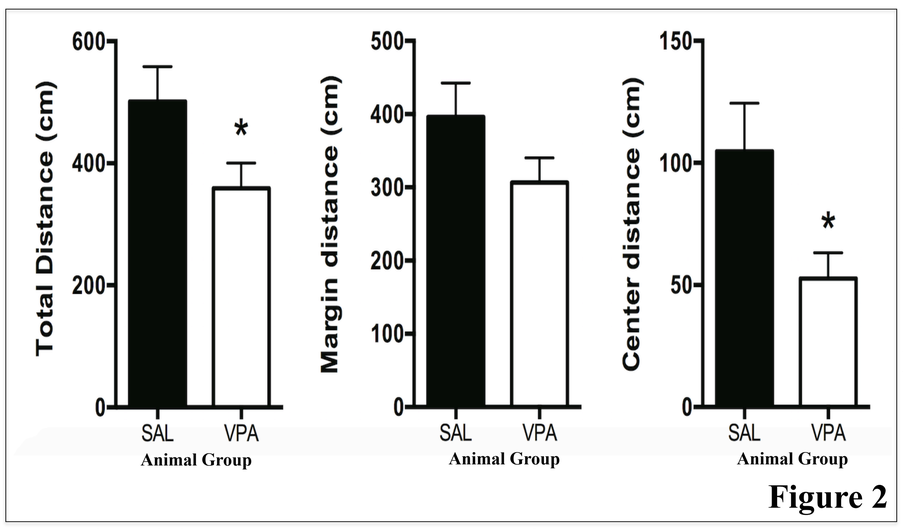

Figure 2

Figure 2 exhibits column graphs generated from the data recorded during the open field test. *Significant.

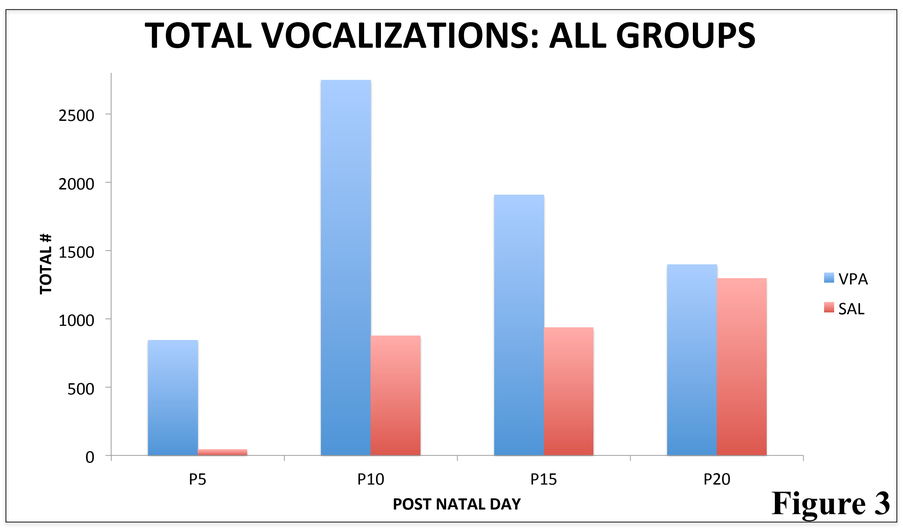

Figure 3

Figure 3 is a column graph generated from the data from the Ultra Sonic Vocalization tests which represents the total number of vocalizations for VPA treated animals and the saline control group.

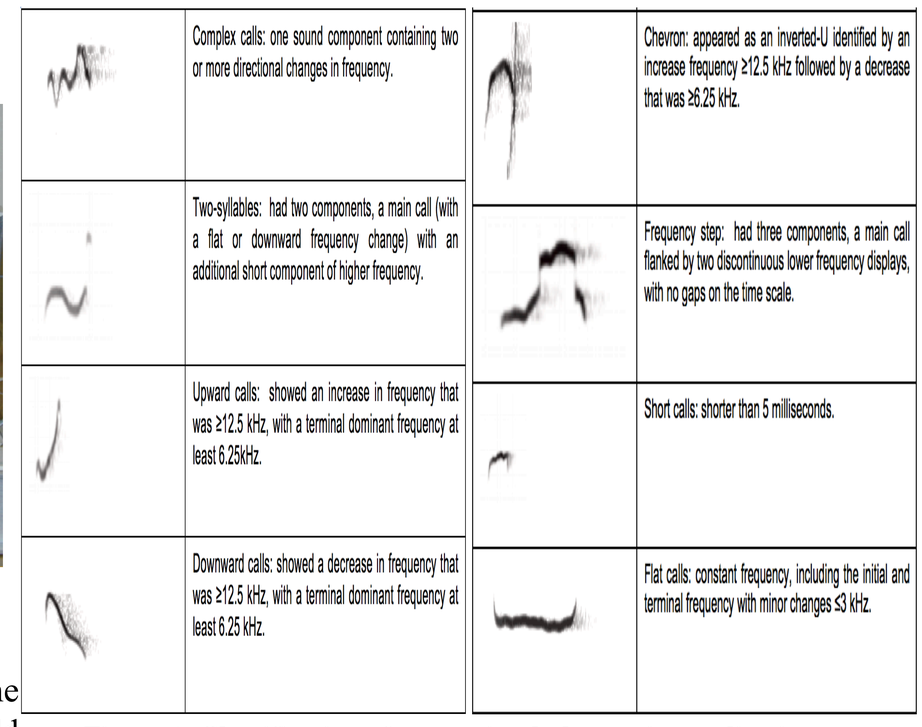

Figure 4: Classification of pup emitted ultrasonic vocalizations.

Conclusions and Future Directions

The aim of this study was to confirm the validity of prenatal exposure to VPA as an ASD rat model. Deficits in the VPA rats include social and behavioral challenges that led to an increase in anxiety levels. This study exhibited that the VPA subjects explored significantly less of the open field and since this test was in a novel arena we have interpreted these results as an indication of increased anxiety levels. This study also showed that prenatal exposure to VPA leads to a dramatic increase in total vocalizations during early development. Since these pups only vocalize when they feel uncomfortable, whether that mean hungry, cold, or feeling separation anxiety, we hypothesize that the exposure to VPA caused an increase in anxiety levels. These indications confirm the literature and indicate that prenatal exposure to VPA is can be a reliable rat model of ASD. Future studies which the Febo lab is currently working on, such as agression tests and fMRI scanning of the rat model, can help determine whether this rat model will have some effectiveness in studying the disease.. Further, these and future results may allow for a deeper understanding of ASD and potentially uncover new ways to prevent, or better yet treat, the social and behavioral deficits characteristic of ASD.

References

Febo M, Jimenez-Rivera CA, Segarra AC. 2002. Estrogen and opioids interact to modulate the locomotor response to cocaine in the female rat. Brain Res 943:151–161.

Felix-Ortiz AC, Febo M (2012) Gestational Valproate Alters BOLD Activation in Response to Complex Social and Primary Sensory Stimuli. PLoS ONE 7(5):e37313. doi: 10.1371/journal.pone.0037313

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