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Level of Expression of BDNF and TNF-Alpha in the Somatosensory Cortex and its Effect on Social Interaction in Autism Model Rats Induced by Valproic Acid

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Abstract

Studies have shown that exposure to valproic acid (VPA) during pregnancy may result in "fetal valproate syndrome" that has the characteristics similar to that of autism. The aim of this study was to investigate the expression of brain-derived neurotrophic factor, alpha tumor necrosis factor alpha and the impact of these factors on the cerebral cortex in autism model rats induced by valproic acid in prenatal period as well as the social functions and behavioral disorders associated with autism animal models of rats. In the middle of the twelfth day of pregnancy (ED 12.5), 500 of mg/kg valproic acid (VPA, SIGMA) dissolved in phosphate buffered saline (PBS) were injected intraperitoneally to female rats in the test group (n = 10). In 30 and 60 days after birth, to evaluate social behavior and foraging activities, social interaction, open field tests and Y-Maze tests were conducted. Then, the expression level of serum and BDNF somatosensory cortex and TNF-α were measured by ELISA method. The results of these tests showed that in autism model rats induced by valproic acid, there are weak social connections, followed by a decrease and an increase in cytokines such as BDNF and TNF-alpha in somatosensory cortex and blood serum of the rats. Such analyses at least in animal models is a way to recognize the social disruption caused by autism.

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Introduction:

Autism refers to child's inability to communicate with people or situations (Gericke et al., 2006). Autism impairs social interaction, verbal and nonverbal communication, and stereotyped behaviors and interests and shows children with these disorders in different categories based on its degrees (Iwata et al., 2010). Diagnosis is done only according to standards of behavior (Willemsen et al., 2000). Autism is a neurodevelopmental disorder that impairs brain function and affects feelings, emotions and memory. Since the disease occurs from the very infancy, it is considered as developmental. Genetics, neurotoxins, neurological diseases are among factors affecting the incidence of autism. Parts of the brain involved in autism include cerebellum and thalamus and hypothalamus, brain stem and cerebral cortex (Duch et al., 2011; Lynch et al, 2013). Diagnosis and treatment of neurodevelopmental disorders is difficult and effective treatment methods include advanced drug therapies and treatment programs at home and school (Tetreault et al., 2012).

Autism spectrum disorders or ASD is a pervasive neurodevelopmental disorder that accompanies the patient during their lifetime. Autism is characterized by the difficulty in mixing with other children and establishing social connections, low or lack of eye contact, resigned behavior, inappropriate attachment to various objects, games and repetitive movements, resistance to routine changes, physical hyperactivity or sever inactivity, extreme irritability distress without any apparent reason, echo speech, no apparent insensitivity to pain, and using gestures instead of words (Duch et al, 2011; Lynch et al., 2013). These deficiencies and countless other environmental factors damage the brain and nervous system of infants with autism (Pardo et al., 2005).

Teratogen-induced animal model for autism: the value of animal models lies in showing specific symptoms or pathophysiological disorders with no need to emulate all pathophysiological aspects of the disease. There are many similarities between VPA animal models and autism in humans. The behavioral tests in rats in the

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VPA group indicates changes similar to the symptoms of autism in humans (Halladay et al., 2009). Research has shown that exposure to valproic acid (VPA) which is an anticonvulsant drug cause teratogenic effects on human embryos especially in the first trimester of pregnancy (Rinaldi et al., 2007).
VPA animal model: in rodents, exposure to VPA during the 12th day, that is, when the neural tube closes, causes disorders that are seen in human autism. Therefore, the 12.5th day of pregnancy is a crucial period for induction by VPA in newborn rats that results in the greatest changes in social behavior similar to human autism (Kim et al., 2011; Reynard et al., 2011).
Brain-derived neurotrophic factor (BDNF): Animal studies indicate that BDNF concentration is in close contact with the CNS and it is likely that BDNF concentration in peripheral blood is effective as a biomarker for autism (Mansour et al., 2010). BDNF and NT-3 (neurotrophin-3) is highly expressed in the hippocampus and cerebral cortex structures, and is related to survival and function of several neuronal populations (Yip et al., 2007).
Tumor necrosis factor (TNF-α): is a pro-inflammatory cytokine that plays an important role in mediating cellular response to injury in the central nervous system. Increased levels of expression of this cytokines in cerebrospinal fluid shows its relationship with cerebral and apoptosis damage (Tarkowski et al., 1999).
The association of cerebral cortex to autism: cortex is region of the brain that plays a main role in understanding the senses. Today, research on sensory problems in autism and results obtained based on a questionnaire, patient's history, observations and retrospective video review and primary test methods primary, and review of the strengths and weaknesses of the investigations, have suggested a multi-sensory integration (Iarocci & McDonald, 2006). Most autism research has focused on cognitive symptoms of autism because they are the only features that make autism diagnosis possible. But it noteworthy that the development and implementation of appropriate cognitive processes depend on primary natural processing and autism-related behaviors requires simultaneous information from many sensory areas (Bertone et al., 2005; Belmonte et al, 2006). In MRI studies, it was found that the cortex of the frontal lobe increases in autistic individuals. In some people with autism the loss of parietal lobe volume has been observed (Schumann et al., 2011). Of autistic individuals, some experience seizures in early childhood and some during puberty and this is due to changes in hormone levels or an imbalance in brain cortical circuits. The seizures occure in a range from severe to mild (Rinaldi et al., 2007). Also, VPA affects the oxidative stress by inhibiting the lipid peroxidation and protein oxidation in rat cortical cells. VPA enhances NMDA receptors transition and increases flexibility in cortex (Lombardo et al., 2009). BDNF and NT-3 (neurotrophin-3) is highly expressed in the hippocampus and cerebral cortex structures and is related to survival and function of several neuron populations (Yip et al, 2007).

Literature review: Leo Caner, professor of psychiatry at the Johns Hopkins Children's hospital, is the first person who researched and studied causes of autism (Volker et al., 2009). Bauman and Kemper in the autistic brain anatomical study dealt with thin capillaries, a slight increase in connective tissue in the arachnoid brain and an increase in the brain cells in the frontal area. One of the salient features highlighted in the autopsy investigation of patients with autism is neocortical disorders with evidence of cortical thickening, increased density of nerve cells, irregular patterns, an increase in the number of nerve cells in the first layer and abnormal pyramidal cells (Bauman et al., 2005; Kemper et al., 2005). In reviewing the autopsy of four autistic individuals, Williams (2000) observed the loss of brain cells and tissue overgrowth but they did not result in adjustment disorders. People with autism have a significant increase in the frontal cortical cytokines in the frontal cortical area and cerebrospinal fluid (Tetreault et al., 2012). Sokhadzy compared multiple cerebral cortex of the brain in autistic children compared and observed significant deficiencies in their executive functions, and these deficiencies were found to be associated with certain mechanisms of the frontal cortex is located in prefrontal and frontal areas (Sokhadzy et al., 2013).
Autism animal models induced by VPA was first proposed in 1996 by Patricia Rudyer et al. (Iwata et al., 2010; Markram et al., 2007). Schneider et al. (2005) conducted some experiments on VPA rat model and found that the rats showed a series of behavioral changes similar to autism symptoms such as reduced sensitivity to pain and increased sensitivity to other stimuli causing pain, repetitive stereotyped behaviors, reduced exploratory behavior and reduced movement disorders and social interactions. In 2009, Mac Fabe et al. showed that ICV infusion of propionic acid to adult male rats can cause both behavioral changes as well as changes in the brain that is similar to the symptoms of autism such as epilepsy, increased repetitive and stereotyped behaviors and damage in social behaviors.

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Research objectives and hypotheses: In the current study, the somatosensory cortex of the brain and its relationship to autism as well as the expression of BDNF and TNF-α level in this area of the brain was measured and assessed. The purpose of the study was to investigate the role of somatosensory cortex of the brain in autism and sensory disorders of the brain in autism related to this brain area which has not been studied and addressed in experimental conditions. Since the processing of sensory-motor dysfunction may play an important role in avoiding emotional contact and social and communication disorders, the present study can be a starting point for obtaining information and studying senses and perceptual experiences of people with autism. Because autism is a developmental disorder that begins in childhood and BDNF is a useful and important biomarker in neurological development for autism diagnostic tests, investigating its role in the disease has opened a new research directions for autism researchers and has resulted in the development of effective treatment methods for this disease. Animal models are useful for studying molecules, pathways and neural circuits that are involved in the pathophysiology and etiology of autism and can pave the way for future research into the treatment of this disease.

Research method:

Animals and research groups: in this study, 20 virgin female Sprague Dawley rats, with an approximate age of two months and an average weight of 200 grams were used. In all stages, moral principles, international law and standards of the ethical committees for laboratory animals were taken into consideration. The rats were placed in two groups: those receiving valporic acid with saline (Test Group) and those receiving only normal saline (Vehicle Control) (Bambini-junior et al., 2011).

Prescribing valproic acid-induced autism model: After mating, the first day of pregnancy was determined using a vaginal sampling (Damke et al., 2010). On the 12.5th day of pregnancy, 500 mg/kg of valproic acid (VPA, SIGMA) dissolved in saline was injected intraperitoneally to pregnant female rats in the VPA group. To the pregnant female rats in the control group only the normal saline was injected (Olexova et al., 2013). After the birth of infant rats, they were counted and their weight and the day of opening their eyes were recorded (Tamburella et al., 2012).

Behavioral tests: all behavioral tests to assess the model of autism were conducted at 30 and 60 days after birth. These tests included Social Interaction, Y-Maze, Elevated PlusMaze, HotPlate and Open Field tests (Schneider et al., 2005).

1. Social interaction test: This test was conducted to investigate and compare indicators related to social behaviors and interactions in rats that received VPA and carriers. Of each test and control groups, two rats with almost similar weight were selected and placed in a transparent cage or a white plastic box (cm 40 × 40 × 50) was placed. The indices in this test included studying behaviors such as looking, touching, cleaning each other, sniffing and licking each other's body, number of collisions and duration of hiding inside the pipe in the first 10 minutes of the test (Markram et al., 2008; Schneider et al., 2005).

2.Y-maze test: This test was performed to assess and compare repetitive behaviors in male and female rats in both the test and control groups. In this test, the rats were individually placed in Y-maze arm and allowed to freely try out one of the arms and stay in the arm for a few seconds. Then, the rats were returned to the starting arm and this procedure was repeated. The index intended in this test is the percentage at which rats in each group select the same arms (Schneider et al, 2005; Markram et al, 2008).

3.Open field test: This test was performed to assess and compare foraging behavior in male and female rats in both test and control groups. Open field device was equipped with a video camera connected to a computer to see the rats' behavior. The time allocated to this test is 5 minutes. The index for this test is the frequency of climbing the wall of the device, frequency of passing from the central and side square, and the amount of disposal (Narita et al., 2010).

Blood and serum: 1 week to 10 days after the end of 60 days and the end of the studies of the rats' behavior, to measure levels of BDNF and TNF in blood, blood sampling was performed directly from the heart and blood serum was prepared (Bambini-junior et al., 2011).

Extraction from the tissue: the somatosensory cortex was isolated and tissue was extracted and used to measure the amount of BDNF and TNF of somatosensory brain tissue (Ghasemi et al., 2014).

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Biochemical tests: Serum levels of BDNF and TNF-α protein was measured using ELISA and BDNF kits. This test can be used to measure levels of BDNF and TNF-α in the sensorimotor cortex in rats receiving VPA and the control rats (Mansour et al., 2010; Ricci et al., 2013)

Statistical analysis: SPSS statistical software was used for statistical analysis between the groups. To determine significant differences (P≤0.05) between the groups, analysis of variance (Anova) and Tukey post hoc test and independent samples T-test were used. EXCEL software was used for drawing the charts (Tamburella et al., 2012).

Findings:

The results of the behavioral tests assessing the model:
The results of Y maze test in one month and two months: Comparison of the mean indicated an increase in the level of repetitive behaviors in the test group receiving VPA compared to the control group in one month.

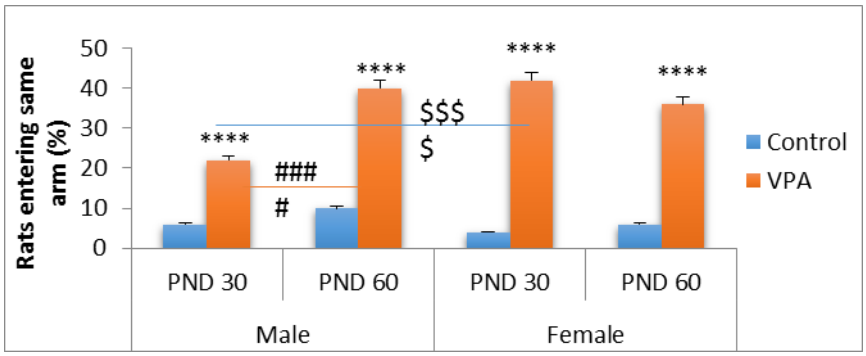

Figure 1. The results of the Y-maze test to measure repetitive behaviors: These results indicate a significant increase in VPA-treated group compared to the control group at both time intervals of 1 month and 2 months of age in both genders (n = 20 in each group) shows (**** p≤0.0001). The effect of gender in a one-month test is significant (**** p≤0.0001) and in relation to age, only in males in the test group receiving VPA a significant difference was observed (**** p≤0.0001).

The results of Open Field test (the study of foraging behavior) in one month and two months old:
Results of the frequency of climbing the walls of the climbing device: comparison of the mean number of climbing the walls of device through independent samples T-test showed a significant difference between the two VPA and control groups in both genders in 30 days and 60 days old. Moreover, the effect of gender was significant in the 30th day but the effect of age was not significant in any of the groups.

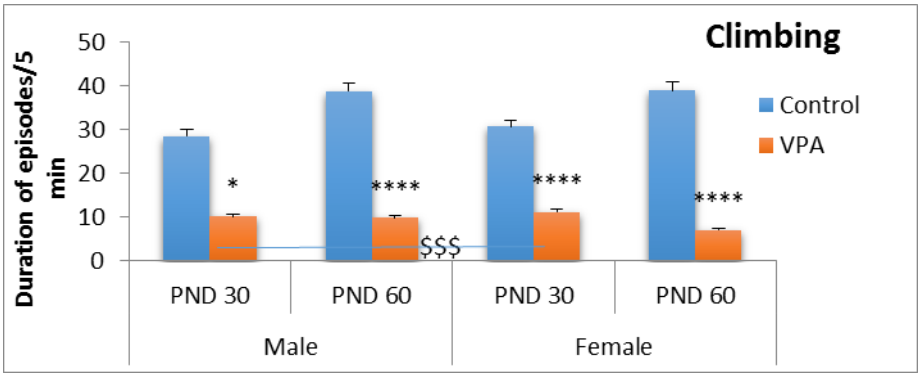

Figure 2. comparison of the climbing index to evaluate the foraging behavior in the Open Field test: Results showed a significant reduction in VPA-treated group compared to the control group at 30 days of age in male rats at p≤0.05 * *** and in female rats at p≤0.0001, and at 60 days of age in both genders at **** p≤0.0001 (n = 20 in each group). In this study, the effect of gender on the 30-day had a significant difference in the test group at *** p≤0.001. The effect of age was not significant in any of the groups.

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Examining the frequency of crossing from the central squares of the Cross to Center: The results showed that a significant difference exists between the two VPA and control groups, in male and female rats at 60th day of age. The impact of gender in the control group was also found as significant at one-month of age in the control group and the effect of age is also significant between male rats in the control group and female rats in the test group.

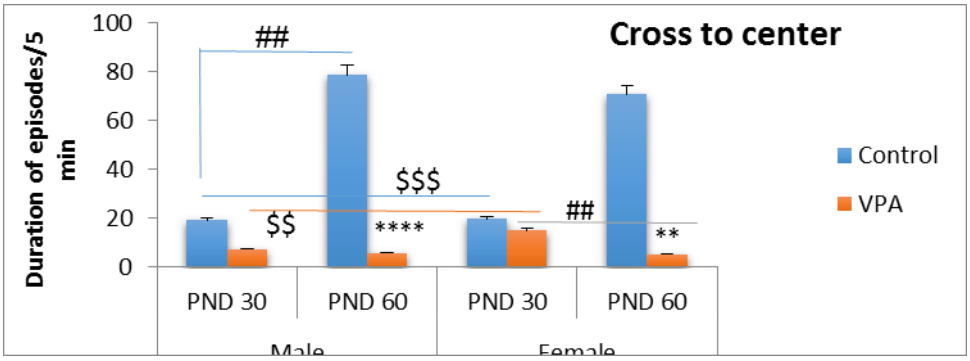

Figure 3. The index of the frequency of crossing from the central squares for assessing the amount of foraging behavior in the Open Field Test. The result indicate a significant reduction in the group receiving VPA compared to the control group in both genders (**** $p \leq 0.0001$) and in females (** $p \leq 0.01$) in the 60th day. The effect of gender in the 30-day control group (** $p \leq 0.01$) and the effect of age on male rats in the control group and test group rats was significant (** $p \leq 0.01$).

Examining the frequency of crossing from the side squares of Crossing: The results showed that there is a significant difference between the VPA and control groups, in female rats and male rats at 30th and 60th days. The effect of gender was not significant in any groups, and the effect of age was statistically significant only in the control group in male and female rats, and insignificant in the group receiving VPA.

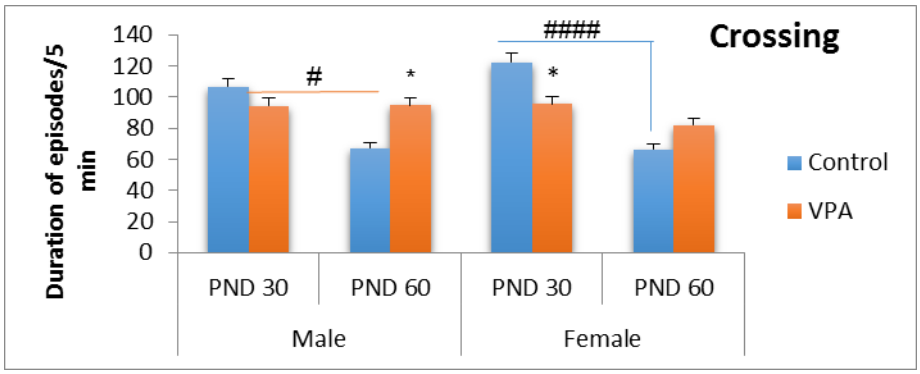

Figure 4. Index of the frequency of crossing from the side squares for assessing the foraging behavior in the Open Field Test. A significant reduction is observed in the group receiving VPA in comparison with the control group in females at the age of 30-days (* $p \leq 0.05$) and in males at the age of 60-days (* $p \leq 0.05$). The effect of age on male rats (* $p \leq 0.05$) and female rats (**** $p \leq 0.0001$) in the control group is significant.

Examining the amount of Stool: According to the results, there is a significant difference between the two VPA and control groups in male rats and female rats at 30th day and at 60th day. In the ages of 1 month and 2 months, the effect of gender is significant in the control group, and the effect of age is only significant in male rats in the control group.

Examining the duration of Freezing Time: The results of the freezing time showed that the two VPA and control groups, in both male and female, in 30-days and 60-days, were significantly different. No statistically significant effect of gender and age is significant only in male rats in the control group.

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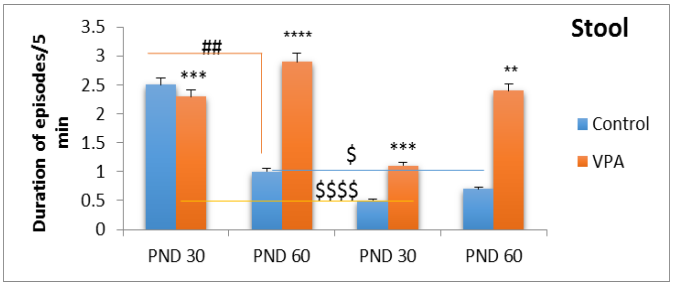

Figure 5. Comparison of the index of the frequency of stools to assess the foraging behavior in the Open Field Test indicated a significant increase in the group receiving VPA compared to the control group in both genders at the age of 30 days (***p≤0.001), and in females at the age of 60 days (**p ≤0.01) (n = 20 in each group). The effect of gender in this study was significant in the 30-days control group (****p≤0.0001), and in the 60-days control group (*p≤0.05). the effect of gender was not significant in any groups, and the effect of age was significant only in male rats in the control group (**p≤0.01).

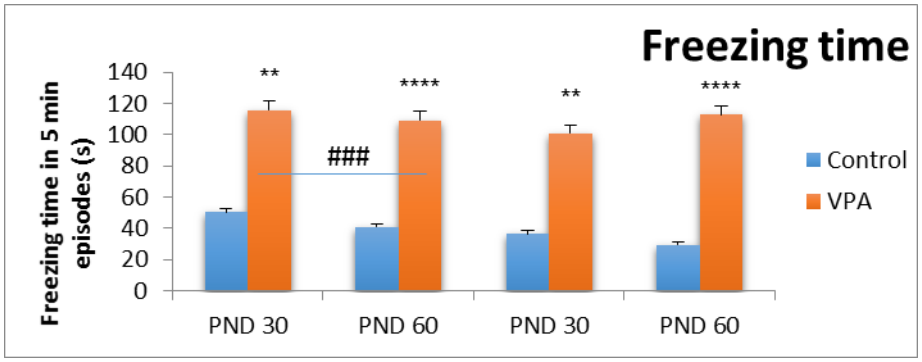

Figure 6. duration of freezing to assess the foraging behavior in the Open Field Test. The results indicated a significant increase in the group receiving VPA compared to the control group in both genders at the age of 30-days (** p≤0.01) and 60-days (**** p≤0.0001). In this study, no significant differences are due to the effect of gender. The effect of age is significant only in male rats in the control group (*** p≤0.001).

Social behaviors and interactions Test:

Results of the Social Interaction test in one-month and two-moths of age: The results of the one-way ANOVA and Tukey table shows that there is no significant difference between male and female rats receiving VPA.

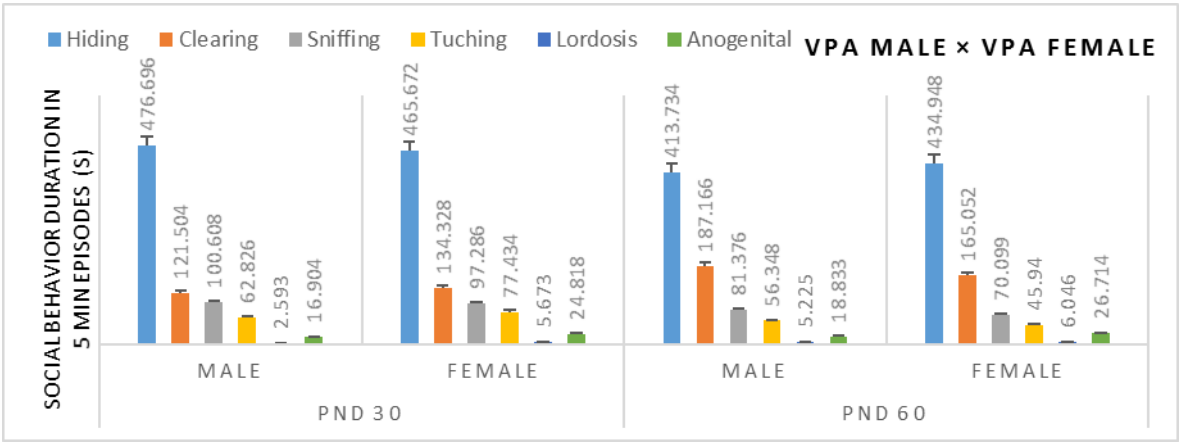

Figure 7. The results of the Social Interaction test to assess the social behavior and interactions: none of the indicators are significantly different.

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Assessing and comparing the indicators of mating between male control rats and female rats receiving VPA: The results are presented as follows: in the index of Hiding, there is a significant difference between the 30-day male rats and 30-days and 60-days female rats, between 30-days male rats and 60-days female rats, and between 30-days female rats and 60-days male rats, and between 60-days male rats and 60-days female rats (****p≤0.0001). In the index of Clearing, there is a significant difference between 30-days male rats and 30-days and 60-days female rats, between 30-day female rats and 60-days male rats, and between 60-days male rats and 60-days female rats (****p≤0.0001). In the index of Sniffing, there is a significant difference between the 30-days male rats and 30-days and 60-days female rats (****p≤0.0001) and 60-days male rats, (**p≤0.01). In the index of Touching, there was a significant difference between the 30-days male rats and at 30-days and 60-days female rats, between 30-days female rats and 60-days male rats, and between 60-days male rats and 60-days female rats (****p≤0.0001). In the index of Lordosis, there was a significant difference between the 30-day male rats and 30-days and 60-days female rats, between 30-day female rats and 60-days male rats, and between 60-days male rats and 60-days female rats (****p≤0.0001). In the index of Anogenital, there is a significant difference between the 30-day male rats and 30-days and 60-days female rats and 60-days male rats (****p≤0.0001), between 30-days female rats and 60-days male rats (**p≤0.01), and between 60-days male rats and 60-days female rats (****p≤0.0001).

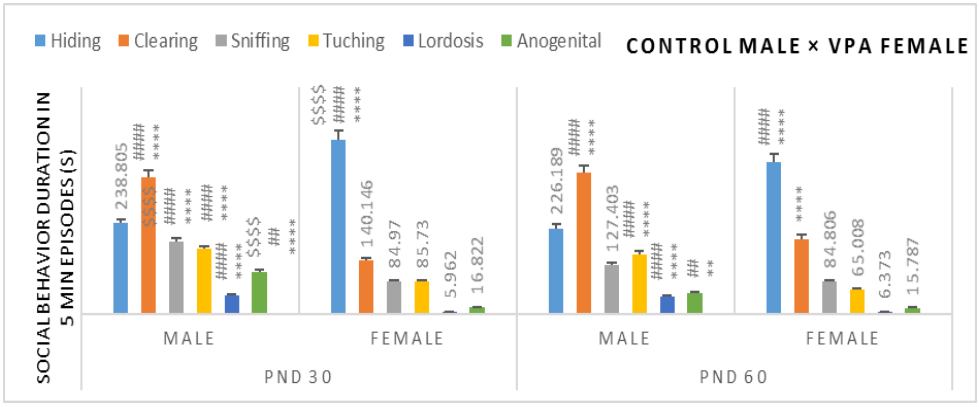

Figure 8. The results of the Social Interaction Test to assess the amount of social behaviors and interactions: in cross breeding between male rats in the control group and female rats receiving VPA. The results of this group is significant in most of the given indexes.

Assessing and comparing the indicators of mating between female control rats and male rats receiving VPA: The results are presented as follows: in the index of Hiding, there is a significant difference between the 30-day male rats and 30-days and 60-days female rats, between 30-day female rats and 60-days male rats, and between 60-days male rats and 60-days female rats (**** p≤0.0001). in the index Clearing, there is a significant difference between 30-days male rats and 30-days and 60-days female rats, between 30-day female rats and 60-days male rats, and between 60-days male rats and 60-days female rats (****p≤0.0001). In the index Sniffing (the amount of sniffing different parts of the body), there is a significant difference between the 30-days male rats and 30-days female rats, between 30-days female rats and 30-days and 60-days male rats (****p ≤0.0001), and between 60-days female rats and 30-days male rats (** p≤0.01). In the index of Touching, there was a significant difference between the 30-days male rats and at 30-days and 60-days female rats, between 30-days female rats and 60-days male rats, and between 60-days male rats and 60-days female rats (****p≤0.0001). In the index of Lordosis, there was a significant difference between the 30-day male rats and 30-days and 60-days female rats, between 30-day female rats and 60-days male rats, and between 60-days male rats and 60-days female rats (**** p≤0.0001). In the index of Anogenital, there is a significant difference between the 30-day male rats and 30-days female rats (****p≤0.0001), between 60-days female rats and 60-days male rats (** p≤0.01), and also between 30-days female rats and 60-days male rats and 60-days female rats, and between 60-days male rats and 60-days female rats (**** p≤0.0001).

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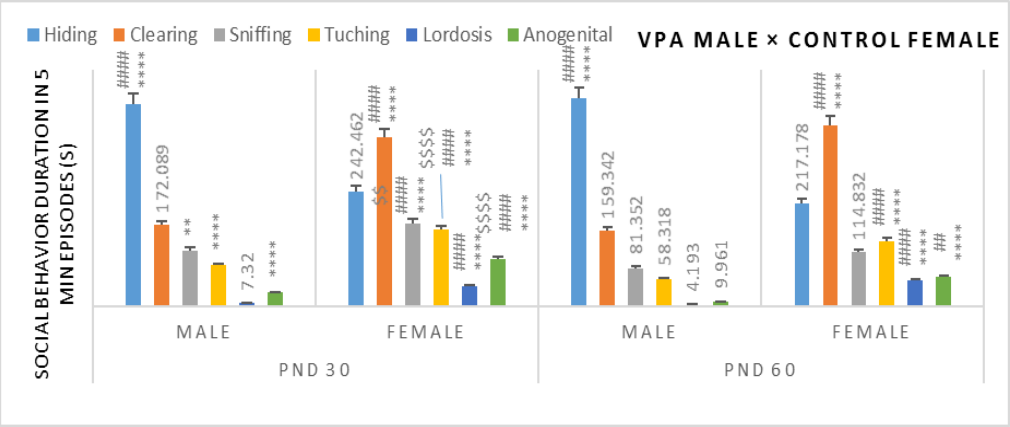

Figure 9. The results obtained from the Social Interaction Test to assess the social behaviors and interactions in cross breeding of female rats in the control group and the male rats receiving VPA. The results showed that there is a significant difference between male rats in the control group and female rats receiving VPA.

Assessing the expression of serum levels of BDNF and the somatosensory cortex level of BDNF: Results of independent t-test indicated that there is a significant difference between the control and the VPA group in serum levels of female rats but the difference was not significant in terms of the somatosensory cortex.

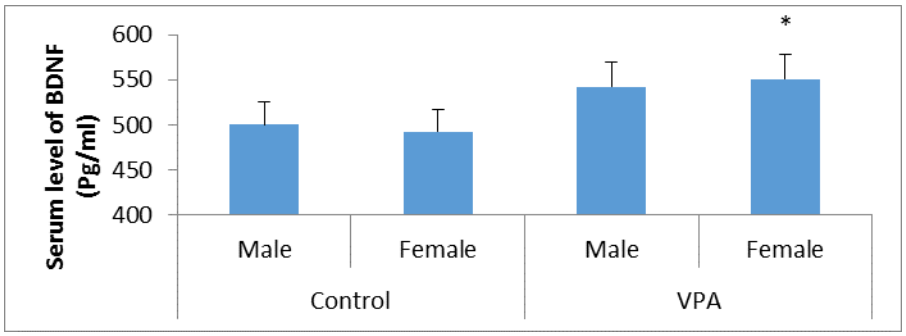

Figure 10. The results of independent t-test for serum levels of brain-derived neurotrophic factor: the results indicated a significant increase in the female rats in the test group than in the control group (* $p \leq 0.05$).

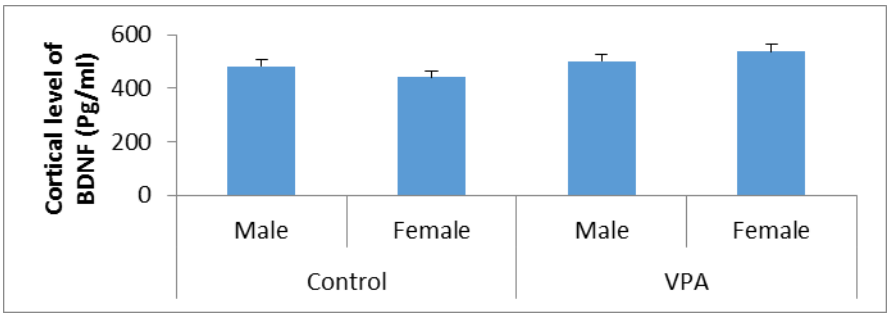

Figure 11. Results of the independent t-test for the somatosensory cortex of the brain-derived neurotrophic factor: The results showed no significant difference.

TNF-α expression in serum levels and the somatosensory cortex: results of independent t-test indicated a significant difference between the control and the VPA in both male and female rats.

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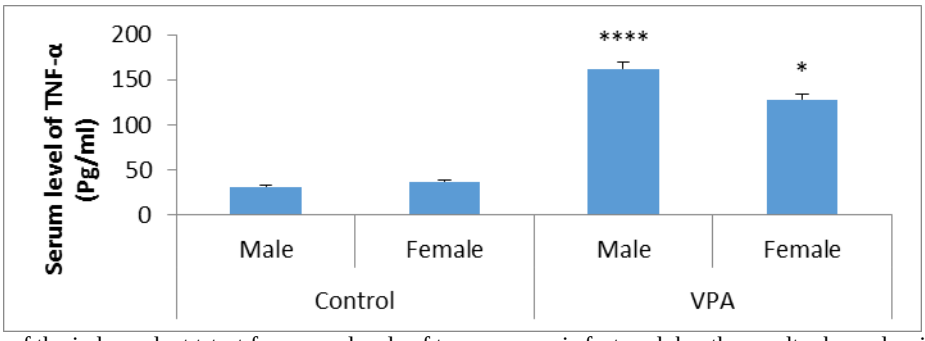

Figure 12. Results of the independent t-test for serum levels of tumor necrosis factor alpha: the results showed a significant increase in both females (* p≤0.05) and males (**** p≤0.001) in the test group compared to the control group.

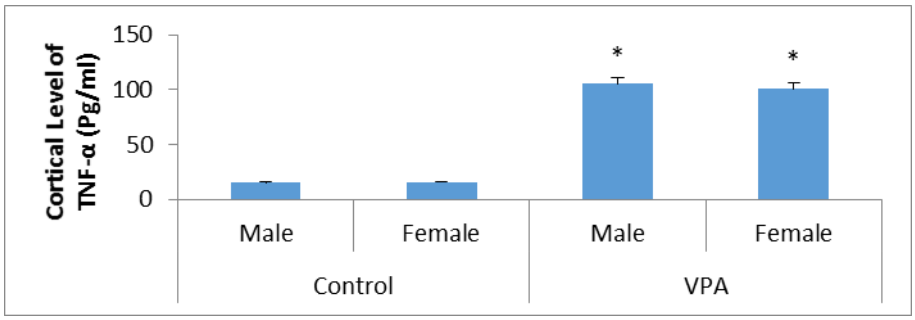

Figure 13. Results of the independent t-test for the somatosensory cortex tumor necrosis factor alpha: the results indicated a significant increase in both genders in the test group compared to the control group (p≤0.05).

Discussion and conclusion:

Models of animals produced by exposure to valproic acid (VPA) during pregnancy has been proposed to study autism which have provided an opportunity to study how environmental challenges that lead to changes in behavior and molecular changes, provide assistance and solutions in the treatment of autism (Rinaldi et al, 2007). It appears that VPA model as an animal model of autism is the closest model for studies and research at all behavioral, genetic, morphological and histological levels. Systematic studies have been carried out at synaptic and behavioral levels (Christianson et al., 1994; Williams & Hersh, 1997; Williams et al., 2001).

In all these studies the relationship between the use of VPA antiepileptic drugs during pregnancy and the risk of having a child with autism has been proved (Wagner et al., 2006). Our results also confirmed many previous behavioral findings in the rats receiving VPA (autistic). The evidence obtained from the VPA rat shows remarkable similarities between animal model of rat and phenotype of autism in humans. The present study also showed that intraperitoneal injection of 500 mg/kg valproic acid (VPA) with normal saline on the day of 12.5th day of pregnancy in female rats caused autism in infants and the produced behavioral modes resulted from the induction of this approach greatly resembles autism in humans. Also, reduced social interaction, increased repetitive behaviors, less sensitivity to pain, sensory and motor dysfunction is seen in VPA children.

Thomas Schneider and Ryszard Przewlocki (2005) presented a model of autism in rodents in which mice were exposed to valproic acid during pregnancy on 12.5th day. The created mice (VPA) had a lot of anatomical and pathological similarities with the human models. However, this new model was not specified in terms of behavior. In order to determine whether the VPA mice also show many behavioral disorders observed in autism, some given behaviors were extensively in a group of tests. Results of the experiments showed that VPA mice are less sensitive to pain and more sensitive to non-painful stimuli. Likewise, repetitive (stereotypical) motions and behaviors such as hyperactivity, decreased social behavior and increased duration of the delay in social behavior, delayed puberty, decreased body weight, and delayed onset of motor behaviors were observed (Schneider & Przewlocki et al, 2005).

The repetitive behaviors: when the newborn rats were one month and two months old, the Y maze test was conducted to evaluate their repetitive behaviors and results achieved by SPSS showed that repetitive and stereotyped behaviors in autistic rats has an increase which is higher in male than female rats.

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These results are consistent with those found by Markram et al. (2007, 2008) and Schneider et al. (2005, 2006). During their examination, in standard conditions, the VPA group had loss of motion and increased repetitive and stereotyped and exploration activities compared to the control group.

The foraging behaviors: To investigate the cognitive impairments induced by VPA, the foraging behavior during the Open Field was studied and rats' movements in an open environment was assessed by an open field test. It was found that a group of VPA rats mostly move in the side squares, they stand on the edge of the device for a short time and the duration of their immobility is longer compared to the control rats. Another group of VPA rats had increased activity and foraging movements. The VPA rats also showed a significant increase in disposal indicating that they had higher anxiety and rats with high anxiety prefer to move in the side square boxes than in the central ones. It was concluded that excessive movements of VPA rats was because of their hyperactivity resulted from high anxiety for being placed in an unfamiliar environment. Likewise, lack of mobility is due to high anxiety in rats (Schneider et al., 2006).
Research conducted over the past few years have shown that other researchers have also found similar patterns. For example, our results are consistent with those of Masaaki Narita et al. (2010) with the creation of an animal model of autism. They concluded high movement of teratogenic rats is due to their hyperactivity which is resulted from high anxiety. Other researchers also found similar results (Markram et al., 2008; Miyazaki et al., 2005; Schneider et al, 2005; Silverman et al, 2010).

Social behaviors and interactions: To investigate the social behaviors and interactions, in one month and two months of age, the Social Interaction test was administered to the newborn rats, and the results obtained by ANOVA and Tukey table indicated that there was a reduction in social interactions and behaviors in VPA rats. The test was administered three times in the male and female test groups, male rats in the control group and female rats in the test group, and female rats in the control group and male rats in the test group, each for 10 minutes. The hiding time inside the tube was an indication of their anxiety and high reduction of social interaction in rats. Other criteria were taken into account in for assessing the level of social interaction included reduction of: sniffing different parts of the body, anogenital sniffing, clearing each other, collisions (lordosis), touching each other, following each other, and also increase in the percentage of hiding inside the pipe. Markram et al. (2008) conducted tests of social behavior and social interactions and found a decrease in VPA rats (Markram et al., 2008).

The relationship between BDNF and TNF-α and autism: animal studies suggest that concentrations of BDNF in CNS and serum are closely correlated and thus make it possible for its concentration in peripheral blood to be a useful biomarker for autism (Karege et al, 2002). During the investigation conducted by Miyazaki et al. (2004) and Hashimoto et al. (2006) found flat surfaces of BDNF in the brain tissue of adults with autism after death. Also, different concentrations were found in the peripheral blood of children and adults diagnosed with autism compared to the control groups (Hashimuto et al., 2006; Miyazaki et al., 2005). In this study, the results of investigating the expression levels of BDNF in the blood and somatosensory cortex tissues showed the elevation of BDNF serum levels reflecting the impact of VPA administered during pregnancy. The elevation of BDNF serum level leads to impairment in the nervous system and the normal development of neonates. The above results are consistent with results of previous studies, but there was no increase in the tissue level in the previous studies.
The results obtained from the analysis of blood and tissue levels of TNF-α in the somatosensory cortex indicated increased serum and tissue levels of TNF-α reflecting the impact of VPA administration during pregnancy. The elevation of serum and tissue TNF-α impairs the immune system and leaves devastating effects that suggests impairment and dysregulation of cytokines in the brains of autistic rats. The results related to serum levels is in line with the results obtained previously by Peterson et al. (2005).

Implications: etiologic studies shed light on effects of administration of valproic acid and its behavioral impacts as a model and extends our understanding of behavioral and brain mechanisms involved in autism and paves the way for further investigation of these studies. Patients with this disease and their families should become familiar with behavioral problems involved in this disorder and the results of this study can provide patients, physicians and research teams with useful information in pre-clinical and clinical studies.

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