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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA

ESTABELECIMENTO DE MODELO ANIMAL DE AUTISMO POR EXPOSIÇÃO PRÉ-NATAL AO ÁCIDO VALPRÓICO:

Parâmetros Comportamentais e Bioquímicos

Victorio Bambini Junior

Orientadora: Prof[a] Dra Carmem Gottfried

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas-Bioquímica da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do grau de Mestre em Bioquímica.

Victorio Bambini Junior

Orientadora: Prof[a] Dra Carmem Gottfried

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Dedico aos meus avós, que me ensinaram os valores que realmente importam.

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"Toda a nossa ciência, comparada com a realidade, é primitiva e infantil - e, no entanto, é a coisa mais preciosa que temos."

Albert Einstein

RESUMO

Autismo é um distúrbio complexo caracterizado por alterações em três principais domínios comportamentais: 1) Interação social; 2) linguagem e comunicação; e 3) limitado número de interesses e atividade. Essa síndrome tem atraído atenção social por sua alta prevalência. Estudos com gêmeos demonstram um forte componente genético, porém fatores ambientais podem ser desencadeadores deste distúrbio. Como resultado destas observações, se estabeleceram modelos animais derivados de exposição pré-natal a xenobióticos. Um dos modelos propostos nos últimos anos para o estudo do autismo utiliza exposição pré-natal em roedores ao Acido Valpróico (VPA). Diversas características comportamentais encontradas no autismo são evidenciadas nesse modelo, tais como atividade repetitiva ou estereotípica, déficit em interação social e ciclo circadiano aberrante. Entretanto, características como rigidez comportamental, memória social e o estado metabólico, ainda não haviam sido estudadas nesses animais. Portanto, o objetivo geral deste trabalho foi estabelecer um modelo animal de autismo com ratos Wistar e especificamente, elucidar novos parâmetros comportamentais que se assemelham ao espectro autista, bem como analisar possíveis danos ou alterações hepáticas que pudessem evidenciar um efeito secundário desse conhecido teratogênico. Com este trabalho evidenciou-se rigidez comportamental nos ratos VPA durante a adolescência. O comportamento social demonstrou-se aberrante tanto na adolescência, quanto na idade adulta, sendo que nesta também se observa uma memória social íntegra e uma busca reduzida por aproximação social por parte dos ratos VPA. Além disso, constatou-se manutenção da integridade hepática. Conclui-se com este trabalho que o modelo estabelecido apresenta as características adequadas para o estudo do autismo, destacando-se rigidez comportamental ou dificuldades de adaptação a uma nova rotina, semelhantes aos portadores de autismo.

ABSTRACT

Autism is a complex disorder charactierized by behavioral impairments in three main domains: 1) social interaction; 2) language, communication and imaginative play; and 3) range of interests and activities. This syndrome has attracted social attention by its high prevalence. Studies with twins show a strong genetic component on autism; however environmental factors can lead the development of this condition. In addition, an animal model to study the autism, induced by prenatal exposure to valproic acid (VPA) has been proposed. Several characteristics of behavioral abnormalities found in the VPA rats, like repetitive/stereotypic-like activity, deficit in social interaction and aberrant circadian rhythm, have parallel in autism. Although, features like behavioral rigidity, social memory and metabolic status of the induced rats still were not observed. Therefore, the focus of this work was contribute to elucidate the behavioral changes occurred by the prenatal exposion to VPA, besides, an liver damage analysis, which could indicate a secondary effect of VPA or even a cause of those aberrant behaviors. The present data indicate that when the VPA rats are young, they show aberrant approach to a stranger rat, decreased conditioned place preference to conspecifics, normal spatial learning and a lack of flexibility on their strategy. When adults, they have shown inappropriate social approach to a stranger rat, decreased preference for social novelty, apparently normal social recognition and no spatial learning deficits. Moreover, those aberrant behavior are not related to hepatic alteration, once the liver have not shown any oxidative damage and the activity of cytoplasmatic hepatic enzymes, found on serum, did not differ between the groups. In conclusion, this established model of study presented characteristics suitable for the study of autism, especially behavioral rigidity or difficulty in adapting to a new routine, similar to individuals with autism.

LISTA DE ABREVIATURAS

SIGLA	SIGNIFICADO
ASD	Desordens do Espectro Autista (do inglês “Autism spectrum disorders”)
CAT	Catalase
MWM	Labirinto aquático de Morris (do inglês, “Morris Water Maze”)
PDD	Transtornos Invasivos do Desenvolvimento (do inglês “Pervasive Developmental Disorders”)
SNC	Sistema Nervoso Central
SOD	Superóxido dismutase
VPA	Ácido valpróico (do Inglês “valproic acid)

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1. INTRODUÇÃO

1.1 Espectro do autismo

Os transtornos do espectro autista, primeiramente descritos em 1943 por Leo Kanner (Kanner 1943), apresentam-se como um distúrbio complexo, definido através de parâmetros comportamentais. O espectro autista é classificado em autista típico, síndrome de Asperger e transtornos invasivos do desenvolvimento não especificados. Nos últimos anos essas síndromes atraíram a atenção pública por sua alta prevalência e elevado custo social (Kogan *et al.* 2009, Rapin & Tuchman 2008).

O autismo é caracterizado por déficits em três domínios comportamentais: 1) interação social; 2) Linguagem, comunicação e processo imaginativo; e 3) limitado repertório de interesses e atividades (Gadia *et al.* 2004, Rapin & Tuchman 2008).

Além disso, existem alguns sintomas associados, como reduzido contato visual, déficit gestual, atrasos de linguagem, inabilidade para interpretar emoções a partir de expressões faciais, hipersensibilidade a estímulos sensoriais, movimentos manuais estereotipados e dificuldade para mudanças em rotinas (Casanova 2007, Geschwind 2009, Rapin & Tuchman 2008). Características adicionais, associadas em alguns casos incluem retardo mental, ansiedade, distúrbios do sono e gastrointestinais, além de maior circunferência craniana e volume cerebral quando jovens (Skoyles 2008, Casanova 2007, Rapin & Tuchman 2008). Apesar de haver um padrão estrito para o diagnóstico, o autismo é uma desordem muito heterogênea. Não existem dois autistas com o mesmo conjunto de sintomas, porém todos os

indivíduos têm déficits na tríade comportamento social, comunicação verbal e não-verbal e comportamentos repetitivos ou estereotipados. Além disso, essas alterações variam em intensidade, mas são presentes ao longo de toda vida (Rapin & Tuchman 2008).

O autismo está presente ao nascimento, todavia usualmente não é diagnosticado até os dois ou três anos de idade. Apesar de existirem sintomas que não podem ser notados nesta idade, como reduzida coordenação motora, muitos pais percebem problemas no progresso social ou comunicativo das crianças. Os déficits sociais não são propriamente claros na infância, porém gradualmente se torna mais evidente (Dover & Le Couteur 2007)

1.2 Epidemiologia

De acordo com os dados epidemiológicos recentes, a densidade de indivíduos afetados por autismo varia entre 1 a cada 166 e 1 a cada 91, um aumento considerável quando comparado com dados de 15-20 anos atrás (Fombonne 2003, Kogan et al. 2009, Fombonne 2009). Por exemplo, entre os anos de 1991 e 1997 essa prevalência teve um aumento de 556%, passando a afetar mais crianças do que, por exemplo, câncer e síndrome de Down (Muhle *et al.* 2004). Esse aumento fez com que as desordens do espectro autista se tornassem uma desordem das mais devastadoras, em termos de prevalência, morbidade, impacto familiar e custo para a sociedade (DiCicco-Bloom *et al.* 2006). Estima-se um gasto médio de 1,6 milhão de dólares por individuo portador de autismo durante a vida (Kogan et al. 2009).

Ao contrário do que chegou a ser cogitado (Wing & Potter 2002), vacinações, pelo uso do coadjuvante timerosal contendo mercúrio, não tem relação com o aumento da incidência de autismo (Fombonne 2008). Não há também qualquer evidência de associação entre autismo, imigração, classe social ou etnicidade (Fombonne 1999). Segundo Fombonne, esse aumento se deve, pelo menos em parte, as mudanças nos critérios de diagnóstico, o que fez com que houvesse uma “migração” de indivíduos com outros transtornos para o autismo (Fombonne 2009). Além disso, estudos epidemiológicos e com modelos animais indicam que fatores ambientais podem ser responsáveis pelos aumentos na incidência de autismo (Fombonne 2003, Schneider & Przewlocki 2005).

1.3 Hereditariedade do autismo

O autismo apresenta um forte componente genético. Estudo com gêmeos apresenta concordância de 60% no autismo clássico em monozigóticos contra 0% em dizigóticos (Muhle et al. 2004). A alta concordância em monozigóticos é um indicativo de que a herança genética é um fator determinante no desencadeamento do autismo. A reavaliação para características mais abrangentes, como déficits em comunicação e alterações sociais, faz essa concordância ir de 60% para 92% em monozigóticos e de 0 para 10% em dizigóticos (Casanova 2006). Uma característica adicional é que homens são 4 vezes mais afetados que mulheres (Casanova 2007). Esses dados sugerem que interações genéticas múltiplas podem ser causadoras do autismo, porém,

fatores ambientais podem contribuir para uma expressão mais saliente dos sintomas (Muhle et al. 2004)..

Observações epidemiológicas sugeriram que teratogênicos poderiam estar relacionados com o desencadeamento do autismo, destacando-se o Acido valpróico (VPA) e a talidomida . Estudos demonstraram que 30% dos fetos submetidos à talidomida entre 20° e o 24° dia de gestação tornaram-se autistas (Miller & Stromland 1999). Entretanto, ao contrário do VPA, a talidomida apresenta diferentes efeitos em primatas e em roedores, sendo que em primatas pode gerar entre outros, crescimento aberrante e deficiente dos membros. Já o ácido valpróico, induz alterações no fechamento do tubo neural observadas similarmente em roedores e seres humanos.

A partir dessas observações sugeriu-se o desenvolvimento de modelos animais para o estudo de autismo, induzido farmacológicamente pela exposição pré-natal ao ácido valpróico (Rodier, 1997).,

1.4 Modelos animais

Estudos com modelos animais fornecem grandes possibilidades de investigações que não são possíveis em humanos, incluindo analises imunológicas e do desenvolvimento, plasticidade e sinalização neural. O autismo é uma condição particularmente difícil de ser mimetizada em roedores. A empatia e a capacidade de intuir os sentimentos e intenções de terceiros, parece ser impossível de ser medida em roedores (Miller 2006). Roedores não podem ser avaliados com déficits de fala e não têm regiões cerebrais diretamente comparáveis com aquelas que medeiam a linguagem humana, habilidade essa extremamente relevante ao estudo do autismo. Por outro lado,

os roedores são em geral, espécies que tem altos índices de interação e comunicação social (Crawley 2003). Essa característica faz com que mudanças no comportamento social de roedores sejam relacionadas a fatores ambientais ou genéticos (Crawley 2003). Devido à alta prevalência do autismo e caráter heterogêneo, um modelo animal torna-se uma ferramenta de valor inestimável no estudo do espectro autista. Dentre os modelos animais existem os induzidos geneticamente, por lesão, microbiologicamente e farmacologicamente (Blaiss *et al.* 2009, Blundell *et al.* 2009, Murcia *et al.* 2005, Rodier *et al.* 1997).

Camundongos *knockout* para ocitocina demonstraram déficit comportamental, concordando com esse sintoma típico do autismo (Winslow & Insel 2002). Hormônios neuropeptideos, como ocitocina e vasopressina, podem mediar o processo de recompensa presente nas interações sociais e estão envolvidos em reconhecimento social em modelos animais (Young 2001). Entretanto, análises não demonstraram uma relação direta entre alterações em genes como o da ocitocina e vasopressina no autismo.

Os modelos induzidos por lesão correspondem a uma parte muito pequena da etiologia do autismo e são mais especificamente utilizados para estudo de fármacos que revertam ou atenuem as condições comportamentais alteradas desses ratos. Os locais de lesões incluem cerebelo, hipocampo e amigdala (Courchesne *et al.* 1995, Piven & Arndt 1995). Porém a baixa reprodutibilidade das lesões faz com que cada modelo tenha caráter quase exclusivo, uma vez que incisões minúsculas podem representar alterações significativas no comportamento animal.

Quanto à indução química, três teratogênicos são tidos como fatores de risco: Talidomida, ácido valpróico (VPA) e etanol (Ingram *et al.* 2000). O modelo animal que vem sendo amplamente utilizado para o estudo de autismo, emprega exposição pré-natal ao VPA (Schneider & Przewlocki 2005). Esse modelo animal apresenta semelhança real com estudos clínicos, que mostram correlação deste fármaco com o surgimento de autismo e foi o modelo eleito para trabalho em nosso laboratório.

1.4.1 Características do modelo animal induzido por VPA, concordantes com o autismo.

Além da etiologia, alterações neuromorfológicas comuns ao roedor induzido e ao portador de autismo já foram demonstradas. Por exemplo, o sistema serotoninérgico é comumente afetado em autistas (Anderson *et al.* 1990). Da mesma forma, a indução em ratos por VPA tem um efeito irreversível na migração e maturação dos neurônios 5-HT positivos (Miyazaki *et al.* 2005)

No cerebelo, mudanças na forma, no número e no volume das células de Purkinje ocorrem tanto em portadores de transtornos invasivos do desenvolvimento como no modelo animal (Ingram et al. 2000). O roedor induzido apresenta também, déficits comportamentais correspondentes aos observados em pacientes, fato esse de extrema importância, visto que o diagnóstico de autismo é dado através da análise comportamental. Alterações no ciclo sono-vigília e diminuição nas interações sociais e nas respostas emocionais são características predominantes no autismo e foram também relatados no modelo animal (Yochum *et al.* 2008, Tsujino *et al.* 2007).

Neuroquimicamente, mudanças na concentração de serotonina também são observadas igualmente em pacientes autistas e no modelo animal. Tsujino e colaboradores demonstraram através de microdiálise, que no córtex frontal de ratos induzidos, os níveis de 5-HT estão cronicamente elevados (Tsujino et al. 2007). Desse modo fica claro que, juntamente com comportamentos aberrantes e com a neuromorfologia do SNC, a serotonina tem sua função alterada pela injeção única de VPA. Porém, o mecanismo exato que desencadeia esses fenômenos no SNC não é claro nem no modelo animal nem nas pessoas com autismo.

Além desses, um grande número de estudos relatam o impacto da exposição pré-natal ao VPA em ratos e camundongos. Entre os efeitos observados estão: retardado no crescimento (massa corporal) e do desenvolvimento (abertura dos olhos) dos ratos VPA quando comparado ao controle (Schneider & Przewlocki 2005), déficit na discriminação olfativa (Schneider & Przewlocki 2005, Schneider *et al.* 2006), menor reação a dor (Schneider *et al.* 2008), atividade exploratória aumentada em campo aberto (Schneider et al. 2008, Tsujino et al. 2007), padrões comportamentais estereotipados aumentados (Schneider et al. 2008), , comportamentos do tipo ansioso aumentados (Zhu *et al.* , Schneider et al. 2008, Markram *et al.* 2008), aumento da memória de tarefas aversivas (Markram et al. 2008) e déficits sociais (Markram et al. 2008, Schneider & Przewlocki 2005)

Embora o modelo de autismo por exposição pré-natal ao VPA esteja bem consolidado como ferramenta para o estudo da etiologia, ainda são necessários estudos relacionados a parâmetros comportamentais. Existem

comportamentos característicos do autismo que ainda não foram pesquisados no modelo, como por exemplo, rigidez comportamental e efeito em tecidos periféricos. E por ser um agente teratogênico, análises complementares do efeito sistêmico do VPA devem ser realizados.

2. OBJETIVOS

2.1. Objetivo Geral

Estabelecer o modelo de autismo em ratos, induzido por exposição pré-natal ao acido valpróico, verificando através das conhecidas mudanças em padrões comportamentais, o desenvolvimento de características autistas.

2.2. Objetivos Específicos.

- Avaliar parâmetros comportamentais para o estabelecimento do modelo, tais como: Teste de sociabilidade no aparelho de três câmaras; teste de aprendizado e medida de rigidez comportamental pelo labirinto em Y (*Y-maze*) e teste de memória espacial no labirinto aquático de Morris (*Morris Water Maze*).
- Avaliar parâmetros bioquímicos relacionados com integridade hepática e com estresse oxidativo (enzimas antioxidantes e dano em lipídeos e proteínas).

3. CAPÍTULO I

Manuscrito a ser submetido para a revista *Neuropsychology*

BEHAVIORAL RIGIDITY IN RATS AFTER PRENATAL EXPOSURE TO VALPROIC ACID

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Abstract

Autism is a complex disorder characterized by behavioral impairments in three main domains: 1) social interaction; 2) language, communication and imaginative play; and 3) range of interests and activities. This syndrome has attracted social attention by its high prevalence. The animal model induced by prenatal exposure to valproic acid (VPA) has been proposed to study autism. Several characteristics of behavioral abnormalities found in the VPA rats, like repetitive/stereotypic-like activity, deficit in social interaction and aberrant circadian rhythm, have been correlated with autism. Features like behavioral rigidity, social memory and metabolic status of the induced rats still were not studied. Thus, the main aim of this work was to investigate additional behavioral rodent similarities with autism as well as possible metabolic dysfunctions caused by the prenatal exposure to VPA, including variations in the redox state of the liver. The present data shows that when the VPA rats are young, they show aberrant approach to a stranger rat, decreased conditioned place preference to conspecifics, normal spatial learning and a lack of flexibility on their strategy. When adults, they have shown inappropriate social approach to a stranger rat, decreased preference for social novelty, apparently normal social recognition, no spatial learning deficits and attenuated resistance to change on MWM. In summary, rats prenatally exposed to VPA presented behavioral rigidity when young, and social impairments correlated to autistic people. In addition, no hepatotoxicity was observed.

Keywords: Autism spectrum disorder, sodium valproate, animal model, behavioral impairments.

1. Introduction:

Autism spectrum disorder (ASD), first described in 1943 by Leo Kanner (Kanner, 1943), is a complex, behaviorally defined disorder of the immature brain comprising autistic disorder, Asperger disorder and pervasive developmental disorder not otherwise specified (PDD-NOS) (Kogan et al., 2009). In the last years, this syndrome has attracted public attention by its high prevalence (Stokstad, 2001). A recent study shows that the prevalence of parent-reported diagnosis of ASD among US children aged 3 to 17 years was estimated to be 110 in 10000 (1 in 91) (Kogan et al., 2009). This data implies high impact on the family, and cost to society (DiCicco-Bloom et al., 2006).

Autism is characterized by impairments in three main domains: 1) social interaction; 2) language, communication and imaginative play; and 3) range of interests and activities (Gadia, Tuchman, & Rotta, 2004; Rapin & Tuchman, 2008). An additional characteristic of the disorder is that three to four times more males are affected than females (Fombonne, 1999). Besides studies with twins show a strong genetic component in ASD and multiple interacting genetic factors as the main causative determinants of autism (Muhle, Trentacoste, & Rapin, 2004) the etiology remains unknown. However, in addition to the genetic predisposition, epidemiological studies have been indicating that is necessary to interact genetic factors with exposure to environmental neurotoxicants (Fombonne, 2003; Wagner, Reuhl, Cheh, McRae, & Halladay, 2006), e.g. prenatal exposure to xenobiotics, such as thalidomide and 2-propylpentanoate/valproic acid (VPA). Thalidomide has been shown to lead to a high rate of autism when exposure occurs during the 20th

to 24th days of gestation (Rodier, Ingram, Tisdale, & Croog, 1997). VPA was recognized as a clinical link to autism characterized by phenotypic abnormalities of the face and developmental disabilities (Christianson, Chesler, & Kromberg, 1994).

Those clinical studies gradually conducted to the development of a few pharmacological animal model of autism by the prenatal exposure to the neurotoxicants (Blaiss, Blundell, Etherton, Sudhof, & Powell, 2009; Murcia, Gulden, & Herrup, 2005; Rodier et al., 1997; Schneider & Przewlocki, 2005; Wagner et al., 2006). Studies with animal models open a great range of possibilities, such as developmental, behavioral and immunological analyses which are not possible to be done with human. By this point of view, animal models of autism would be an invaluable tool, allowing further studies about subjects impossible to be analyzed by other resources (van der Worp et al., 2010). The teratogenic potential of VPA during pregnancy and its consequences during the human and rodent neurogenesis have been studied (Christianson et al., 1994; Rodier et al., 1997). A single administration of VPA in uterus, leads to developmental delays and lifelong deficits in motor performance, social behavior, and anxiety-like behavior in the offspring (Kolozsi, Mackenzie, Roullet, Decatanzaro, & Foster, 2009; Schneider & Przewlocki, 2005), including developmental, anatomical and functional similarities with human autistic patients (Rodier et al., 1997; Schneider, Turczak, & Przewlocki, 2006). Neuron counts in rats exposed to VPA showed reductions of cell numbers in the cranial nerve motor nuclei and also had cerebellar anomalies like those reported in studies of autistic cases, supporting the idea that these animals may be

a useful model of the developmental injury that initiates autism (Rodier et al., 1997)..

Although a significant number of studies show the consequences of prenatal exposure to VPA at central nervous system level (Rodier et al., 1997; Tsujino et al., 2007), more investigations are needed to clarify if any other areas are also affected. The cell redox system and the damage induced by it could be an indicative of oxidative stress associated to liver disease. Thus, the main aim of this work was to investigate additional behavioral rodent similarities with autism as well as possible metabolic dysfunctions caused by the prenatal exposure to VPA, including variations in the redox state of the liver.

2. Material and Methods

2.1 Animals

Experiments were performed according to the *NIH Guide for the Care and Use of Laboratory Animals* and approved by local authorities. Female Wistar rats coming from the local breeding colony (ICBS-Federal University of Rio Grande do Sul), with 12:12 light cycle (lights on at 7:00 and lights off at 19:00), with controlled temperature (22 ±1^{o}C) and with water and food ad libitum, had their fertility cycle controlled, and, when on proestrus, mated overnight (Marcondes, Bianchi, & Tanno, 2002). If spermatozoa were found in the morning, it was designated as first day of pregnancy. Valproic acid (Acros Organics, New Jersey, USA) was purchased as the sodium salt and dissolved in 0.9% saline for a concentration of 250 mg/ml. Females received a single intraperitoneal injection of 600 mg/kg sodium valproate (VPA) or physiological saline (control) on E12.5 (Schneider &

Przewlocki, 2005). As described previously, the delivery of this dose results in maximum levels of total VPA (900 µg/ml) in maternal plasma in less than 1 h, with a mean/average plasma elimination half life of 2.3 h (Binkerd, Rowland, Nau, & Hendrickx, 1988). Females were kept separate and with free access to their own litters. Rats from both groups control and VPA were born healthy and the number of offspring was normal. The offspring rats were weaned at 15 days old and were housed separately by sex. The experiments were performed twice, using 4-6 male rats from each litter. Rats had free access to food and water. All the experiments were performed between 12:00 and 17:00. In way to analyze the differences between young and adult rats. Behavioral tests were performed at 35-50 and 80-115 days old, considered young and adult rats, respectively. At 120 days old, rats were sacrificed; the tissues were removed and kept in -70^{o}C until the assays were performed.

2.2. Behavioral tests

Three-chamber sociability test: The social test was performed on a three chambered apparatus as described previously in a mice task, with modifications required to perform it in rats (Nadler et al., 2004). It is a wooden box with partitions separating the box into three chambers. The openings between compartments allowed free exploration to the different chambers. Time spent in each chamber; as well the time spent exploring the “strange” rat or an object into the chamber, was analyzed by two observers. The object was an empty identical cage used to enclose the “strange” rat. Chambers were cleaned with 70% ethanol and water between tests. Animals used as “strangers” were Wistar males with the same age

and no previous contact with the test rats. Animals were individually acclimated for 5 minutes into the apparatus on the day before the experiment. On the sociability test, rats were allowed to expend 5 minutes on the central chamber, and then the stranger rat was introduced into one of the side chambers. When performed with young rats (40-45 days old), the experiment was performed in up to 10 minutes, with the stranger rat and with an object on each side. When performed with adult rats (100-110 days old), a 10-minute test to quantify preference for social novelty began immediately after the 10-minute test for sociability. On this test, the original "stranger" rat (stranger 1) remained in its wire cage on one side of the apparatus and a new unfamiliar rat (stranger 2) was placed in the wire cage on the opposite side, which was previously empty during the sociability test. The score was evaluated by the time spent in each chamber and by the time spent sniffing each wire cage. Stranger 1 and stranger 2 animals originated from different home cages had never been in physical contact with the subject rat. The three chambered apparatus was centered onto a lab bench to minimize light-gradients in, temperature, sound and other environmental conditions that could produce a side preference.

Y-Maze: Learning ability was tested as described (Aguilar, Minarro, & Felipo, 2000; Piedrafita, Erceg, Cauli, Monfort, & Felipo, 2008) in a wooden Y-shaped maze, which had three arms of equal size (60 cm long, 11.5 cm wide and 25 cm high). The arm where the rats were placed at the beginning of each trial was considered the start arm. The other arms, each of which had a food cup located at the end, were considered the choice arms. Pre-training was carried out during 4

days with all rats to familiarize them with the maze. In each trial, rats were rewarded for choosing the left arm. The reward for the correct response consisted of half Froot-Loop® placed in the food cup at the end of the correct arm. If the rat made an incorrect choice, it was allowed to go to the empty food cup at the end of the incorrect arm and was removed after 5 sec. Rats were trained for ten trials per day, with an intertrial interval of approximately 5 min in their home cage until the completion of a criterion of ten correct responses in ten consecutive trials or until a maximum of 250 trials. After the subject reach the criterion, the reward was placed at the end of the reverse arm and the number of trials was measured until the rat changed its strategy, by choosing the other arm (the right side one).

***Spatial Learning in the Morris Water Maze (MWM)*:** The MWM, an established test of spatial learning and reference memory, was conducted in a 210 cm in diameter tank made of fiberglass and painted flat black (Vorhees, Johnson, Burns, & Williams, 2009). The 3 walls nearest to the maze (representing arbitrarily N, E, and W walls of the test room) had large geometric figures mounted above the edge of the pool. The experimenter stood at the “S” position during testing and remained stationary. Testing was conducted in 2 phases, acquisition and reversal. Each phase consisted of 4 trials/day for 5 consecutive days; this was followed by one additional day when a single probe trial was given. Probe trials lasted for 30s. The time limit on learning trials was 1 min and the intertrial interval (ITI) was 5 minutes spent on the cage. Animals who did not find the platform within 1 min were placed on the platform again. Goal platforms (with diameters of 10 or 7 cm) were made of black acrylic with thin nylon screening attached to the surface to

provide traction. The platform was positioned 1–2 cm below the surface of the water and was camouflaged by virtue of being transparent against a black background. Water temperature was 21±1°C. The acquisition phase occurred on P81–86. During acquisition, the 10 cm diameter platform was used and located in the SW quadrant halfway between the center and the side of the tank. Rats started at one of four positions located distal to the quadrant containing the platform in a random order with the restraint that they received one trial from each of the four starting positions per day. The start positions used were: NW, N, E, and SE. These positions were used to eliminate short paths to the goal, such as those that are possible if S or W starts are used. The day after the last acquisition trial, each rat was given a 30 sec probe trial. For the probe trial, rats were started from a position they had never been started from before (NE). On P88–93 rats were tested in the reversal phase with a 7 cm diameter platform placed in the NE quadrant. The smaller platform was used to increase the spatial accuracy required to locate the platform. The same procedure was used as for acquisition (5 days, 4 trials/day). Start positions were SE, S, W, and NW. On the sixth day, the platform was removed and a 30 sec reversal probe trial was administered with the start position at SW.

2.3 Biochemical blood parameters and oxidative stress analysis

Blood sampling and analysis: At 120 days-old, overnight-starved animals (6^{th} hour) were anesthetized by intramuscular injection of 75 mg/kg ketamine and 10 mg/kg of xylazine, respectively. Blood samples were obtained by intracardiac punction and animals were killed by decapitation. Blood samples were incubated at

room temperature (25°C) for 5 min and centrifuged at 3200 rpm for 5 min. Serum was stored at -70°C until the day of analysis. Biochemical analyses of ALT/GPT (alanine aminotransferase or glutamic-pyruvic transaminase) and AST/GOT (aspartate aminotransferase or glutamic-oxaloacetic transaminase) activity were performed using kits from Human do Brasil.

Tiobarbituric acid reactive species (TBARS) assay: As an index of lipid peroxidation, TBARS formation was measured using a hot acid reaction. The homogenates of rat liver slices were mixed with 0.6 ml of 10% trichloroacetic acid (TCA) and 0.5 ml of 0.67% thiobarbituric acid, and heated in boiling water for 25 min (Draper & Hadley, 1990). The levels of TBARS were spectrophotometrically determined at 532 nm. Results are expressed as nmol MDA equivalents/mg protein.

Oxidative alterations in proteins measured by sulfhydryl content: A sample aliquot was diluted in 0.1% SDS and 10 mM 5,5-dithiobis 2-nitrobenzoic acid (DTNB, Sigma). Ethanol was added to produce the intense yellowish color of the product of the reaction between the sulfhydryl (-SH) groups and DTNB. After 20 min, -SH levels were spectrophotometrically determined at 412 nm (Ellman, 1959). Results are expressed as µmol (-SH).mg protein^{-1}.

Determination of carbonil groups: Protein carbonyl formation was measured as an index of protein oxidative damage (Levine et al., 1990). This method is based on the reaction of dinitrophenylhydrazine with protein carbonyl groups. The results are expressed as µmol.mg protein^{-1}.

Superoxid Dismutase (SOD) activity assay: SOD activity was quantified by the inhibition of superoxide-dependent adrenaline auto-oxidation to adrenochrome using spectrophotometric measurements at 480 nm, as previously described (Misra & Fridovich, 1972). The results are expressed as absorbance/time (sec). The area under the curve of the graph was used for statistical analysis and compared against the control values. Five units of CuZnSOD (E.C.: 1.15.1.1) were used to determine assay specificity.

Catalase (CAT) activity assay: CAT (E.C.:1.11.1.6) activity was assayed as previously described (Aebi, 1984) by measuring the absorbance decrease at 240 nm in a reaction medium containing 20 mM H_2O_2, 0.1% Triton X-100, 10 mM potassium phosphate buffer, pH 7.0, and 50 µg protein. One unit (U) of the enzyme is defined as 1 µmol of H_2O_2 consumed per minute and the specific activity is reported as U/mg protein.

Statistical analysis: Data are reported as mean ± standard error mean (S.E.M.) and were analyzed by Student's t-test. Values of $P< 0.05$ were considered significant. All analyses were performed using the SPSS program, Version 12.0 (SPSS, Chicago, IL).

3. Results

3.1 Behavioral testing:

3.1.1. Young rats

Three-chamber sociability test: The time spent in the chamber 1, which contains the stranger rat, was 349.2 ±15.7 sec to the control and 305.1±51.3 to the VPA group. On the chamber 2, which contains the object, the time spent by the

control rats was 167.5±13.4 and the VPAs' 191.7±44.29. On the central chamber 83.2±9.9 to the control rats and 103.2±10.0 to the VPA rats. Both groups spent less time in the central chamber than in the chambers 1 and 2. Control group spent more time in chamber 1 than on chamber 2 (p<0.05) and VPA group did not show this difference.

A significant effect of group was observed on the time spent exploring the stranger rat, 180.5±26.6 sec to the control and 66.1±15.4 sec to the VPA group. Means comparisons revealed that VPA rats explored less the stranger rat than control males (p<0.001). The time exploring the object has no difference between the groups (72.0±12.5 to the control and 46.6±8.4 to the VPA).

Y-Maze: No statistical difference was found in the learning ability, expressed by the number of missed trials until they learn the task between VPA (12.76±2.11) and control (12.93±2.19) groups. However, there was a significantly 2-times increase in the number of trials on the reversed Y-maze, showing persistence onto the sameness on VPA (3.37±0.9) group compared to control (1.67±0.12).

3.1.2. Adult rats

Three-chamber sociability test: The first phase of the experiment shows statistically decrease (22%) in the time spent exploring the stranger rat by VPA (116.8 ±8.7) compared to control group (150.8±11.3, p<0.05).

On the second phase, the time spent in the chamber 1, which contains the first stranger, was statistically different between groups (168.8±13.1 sec control and 224.5±16.8 sec VPA, p<0.05). Besides, a difference on the time spent exploring a

new strange rat was also found, showing a lower interest to begin a social approach on the VPA group (163.7±18.2 control and 105.6±15.4 VPA, $p<0.05$)

Morris water Maze: No statistical difference was found between the groups VPA and control in the Morris Water Maze and in the Reversed Morris Water Maze

Biochemical analysis: Liver citotoxicity was measured by the serum activity of the hepatic enzyme markers ALST/GOT and ALT/GPT. Prenatal exposure to VPA did not modify the activity of those enzymes compared to control group, indicating that this teratogen did not affect hepatic cell integrity. Oxidative damage, by damage on lipids (TBARS), damage on proteins (-SH and carbonyl content) and the activity of two important antioxidant enzymes, SOD and CAT, had also been measured at liver. The activity of SOD (U SOD.mg protein^{-1}) was not different between control (45.00±3.54) and VPA (44.75±1.92) groups (Figure 5A). However, VPA induced a significantly increase (12%) in CAT activity (from 144.89±7.82 to 169.02±8.9 U CAT.mg protein^{-1}) in VPA group (Figure 5B). The SOD/CAT ratio (Figure 5C) decreased on VPA rats (from 0.310±0.020 to 0.267±0.037). As showed in Figure 6, no statistically significant differences were found on TBARS (from 0.0812±0.0026 to 0.0755±0.0065 nmol TMP.mg protein^{-1}), sulfhydryl (-SH) (from 36.7±5.54 to 36.7±2.28 µmol (-SH).mg protein^{-1}) and carbonyl content (from 2.80±0.74 to 3.56±0.39 µmol carbonyl.mg protein^{-1}).

4. Discussion

The animal model of autism induced by VPA, have a significant number of characteristics observed in autistic people. Among those are repetitive and

stereotypic behavior, decreased number of social behaviors (Schneider et al., 2006), impairment on the initiation of social interaction (Dufour-Rainfray et al., 2010) and abnormality of circadian rhythm (Tsujino et al., 2007).

The present work shows an evaluation of social memory, behavioral rigidity and biochemical parameters. Behavioral tasks at young age showed the following results: (a) a decreased time spent by the VPA rats exploring a stranger rat on three chambered apparatus; (b) While control rats spent more time in chamber 1 than chamber 2 ($p<0.05$), VPA rats did not show any preference; (c) No difference to learn a the Y-Maze task and (d) an increased latency on VPA rats to change their strategy on reversed Y-Maze.

(a) On the three chambered apparatus, the time spent exploring the “strange” rat was significantly lower in the VPA group indicating reduced social approach to a stranger rat. It clearly is a social dysfunction induced by the prenatal exposure to VPA that is a consistent characteristic of autism. Besides, it is shared by the other animal models (Crawley, 2007; DiCicco-Bloom et al., 2006).

(b) Control rats have shown a preference to conspecific, spending more time in the chamber which contains the “stranger” rat than in the chamber with the object *(*$p<0.05$). This typical social rat behavior was not shown by the VPA group, indicating a lack in the preference for a place with a conspecific.

(c) It is well known that mental retardation is a characteristic often related with autism (Casanova, 2007). On the Y-Maze, a spatial discrimination learning task, no difference between the groups were found, which led us to believe that the VPA

rats have no learning impairment, at least on the behavioral task that we have analyzed.

(c-d) Once VPA rats learned the task normally, when they were challenged to try a new strategy, the VPA rats showed a lack of flexibility, spending more trials on the first reward arm. It could be related with the behavioral rigidity, which is very typical and present on autism (Crawley, 2007).

When they were adults, the tests performed were three-chambered apparatus (2 phases) and Morris water maze (acquisition and reversion). The three-chamber apparatus tasks provided a good perspective about how impaired is the social behavior of the rat exposed to VPA on the gestation. The first phase of the paradigm had a statistical significant difference between the groups; VPA group spends less time exploring the “stranger” rat. Therefore, it is a similar type of aberrant behavior prevailing at young ages on VPA rats. Schneider´s group (Schneider et al., 2006), found that the VPA rats in adolescence have a lower number of pinnings, although the latency to pinning and duration of pinning did not differ between groups. Moreover on the second phase of the test, where the preference for social novelty was evaluated, the induced rats showed less interest to make a new social approach. We reach this conclusion, once the time that they spent exploring the new “stranger” rat was significantly lower than the controls.

Nevertheless, the time spent exploring the first “stranger” on the second phase decreased when compared with the first phase ($p<0.05$) to both groups. It means that the VPA rats probably have a normal social recognition, because they showed the same degree of interest (inferred by the time spent exploring) as the controls

for the first strange on the second phase and in addition exploring the new stranger more than the first on the second phase ($p<0.05$). Besides, the VPA group spent more time than the controls in chamber 1 on the second phase. This could be a consequence of the fact that VPA rats were more comfortable in a chamber with a known rat to a chamber in which they will be submissive to a new social approach.

On the MWM task, the VPA rats showed the same behavior that the controls on both acquisition and reversion phases. It is strong evidence indicating that spatial learning and reference memory are still fully functional in the animal model of autism. Besides that, the VPA rats showed a very good adaptation to the new task on the reversal MWM, similar to the controls. It is an evidence that, the inability to change habits which these animals previously shown, were attenuated from young to the adult age (Silva & Ehninger, 2009).

It is of common knowledge that pharmacological tissue damage could led to a huge spectrum of behavioral impairments (Delongeas *et al.*). Accordingly, we must try to elucidate if those aberrant behaviors came selectively from a brain damage or if it came from, at least in part, from other metabolic pathways. It possibly could happen, since the prenatal exposition to the teratogenic VPA is affecting the pups' body globally (Binkerd et al., 1988; Christianson et al., 1994; Ingram, Peckham, Tisdale, & Rodier, 2000).

Therefore, it seems proper to assume that the liver, an organ that plays a central role on metabolic status, is an important place to look at for possible damage or metabolic alterations. In way to analyze the liver cells' integrity, we quantified the urea content and the activity of the hepatotoxicity cytoplasm

enzymes AST/GOT and ALT/GPT in the serum from these animals. These parameters were not changed between the VPA and control rats (Table 1), consequently indicating absence of hepatic damage.

Besides, we have analyzed oxidative damages which could compromise the cells' function without causing a rupture of plasmatic membrane. No damage was observed on both proteins (by the evaluation of sulfhydryl and carbonyl groups) and lipid membranes (by TBARS). The enzyme SOD dismutates the $-O^{-2}$ generating H_2O_2 which is converted in $2H_2O + O_2$ by catalase. H_2O_2 can act as a signaling molecule or, with transition metals, yield the hydroxyl radical (•OH) (Fenton and Habber-Weiss reaction) (Halliwell, 2006). Thus, we have found an increased CAT activity and decreased SOD/CAT ratio; however, no oxidative damage was observed by the parameters studied. It is an indicative that hepatic cells were able to handle the production of free radical and sub-products.

In summary, the present data indicate that when the VPA rats are young, they show impaired approach to a stranger rat, decreased place preference to conspecifics, normal learning and a lack of flexibility on their strategy. When adults, they have shown inappropriate social approach to a stranger rat, decreased preference for social novelty, apparently normal social recognition, no spatial learning deficits and normal resistance to change on MWM.

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Table 1. Activity of hepatic enzyme markers and urea content in serum from 120 days-old rats prenatally exposed to VPA.

	AST/GOT (IU/L)	ALT/GPT (IU/L)	Urea (mg/dL)
Control	95.36 ± 5.87	46.5 ± 6.53	186.14 ± 10.41
VPA	91.40 ± 4.45	49.2 ± 5.6	183.03 ± 9.79

AST/GOT, aspartate aminotransferase or glutamic-oxaloacetic transaminase; ALT/GPT, alanine aminotransferase or glutamic-pyruvic transaminase. Values are means ±SEM, *n* = 4-6.

Legends of Figures

Figure 1: Three-chamber sociability test in adolescent rats prenatally exposed to VPA. Social approach was measured after 10 min of acclimation and 10 min of test. Chamber is 1 the chamber with the new rat and the chamber 2 contains the object. Data are expressed as mean ±SEM, n=8-12. *different from control group, $p<0.05$.

Figure 2: Learning ability and behavioral rigidity measured in the Y-Maze in adolescent rats prenatally exposed to VPA. A. Learning ability, expressed as the number of wrong trials until learn the task: 10 right response in 10 consecutive trials. **B**. Number of trials until change the strategy. Data are expressed as mean ±SEM, n=8-12. *different from control group, $p<0.05$.

Figure 3: Three-chamber sociability test in adult rats prenatally exposed to VPA. **A**. Phase 1: Sociability measured after 5 min to acclimation and 10 min of test. **B**. Phase 2: Social memory after 10 minutes of test. In the first phase of the task the chamber 1 is the chamber with the “stranger rat 1” and the chamber 2 is the one which contains the object. In the second phase chamber 1 contains the “stranger 1” (present in the phase 1) and the chamber 2 contains a new rat, the “stranger 2”. Data are expressed as mean ±SEM, n=8 per group. *different from control group, $p<0.05$.

Figure 4: Assessment of spatial learning ability determined by the Morris Water maze (MWM) in adult rats prenatally exposed to VPA. **A**. Spatial learning ability. **B**. Ability to change the strategy to learning a new task on the reversed MWM. In both tests, no statistic differences were observed between the control and VPA group, n= 8-12.

Figure 5: Liver antioxidant enzymes activity in adult rats prenatally exposed to VPA. **A**. SOD activity; **B**. CAT activity and C. SOD/CAT ratio. The experiments were performed in triplicate (n=4-6). Data are expressed as mean ±SEM. *different from control group, $p<0.05$.

Figure 6: Oxidative damage parameters in liver from adult rats prenatally exposed to VPA. A. Lipid peroxidation assessed by TBARS; **B.** Protein damage by reduced sulfhydryl content; **C.** Protein damage by carbonyl content. Data are expressed as the mean ± SEM, and experiments were performed in quintuplicate (n = 4-6).

Figure 1

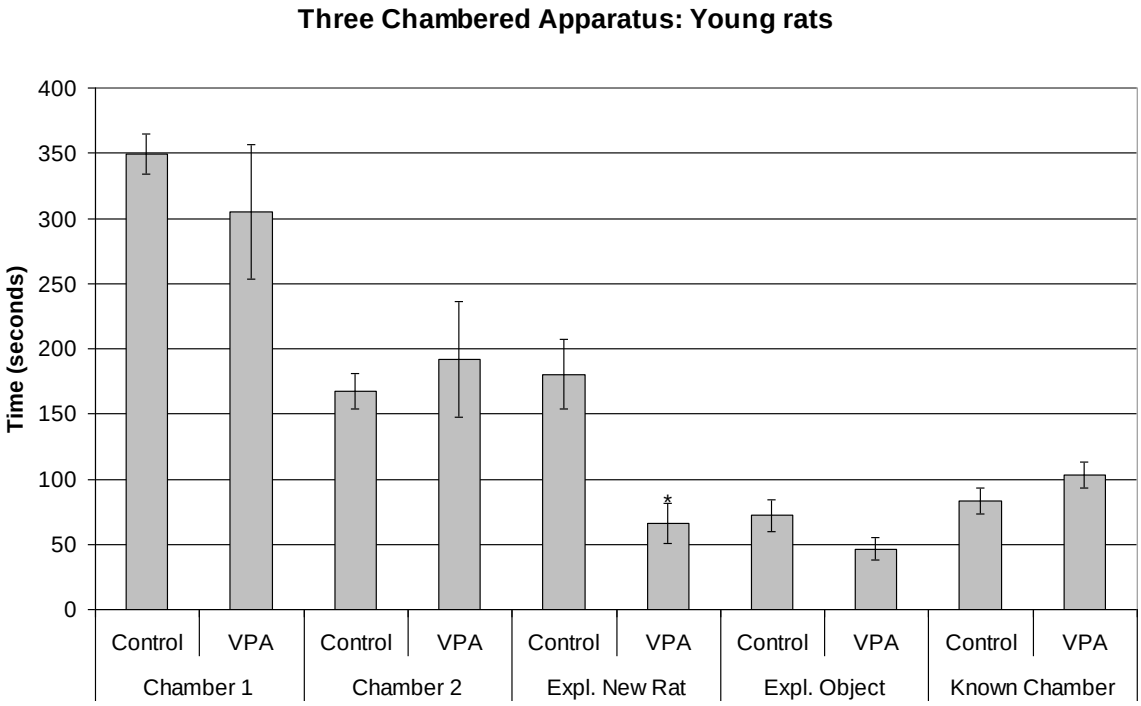

Three Chambered Apparatus: Young rats
Time (seconds)
400
350
300
250
200
150
100
50
0
*
Control
VPA
Chamber 1
Chamber 2
Expl. New Rat
Expl. Object
Known Chamber

Figure 2

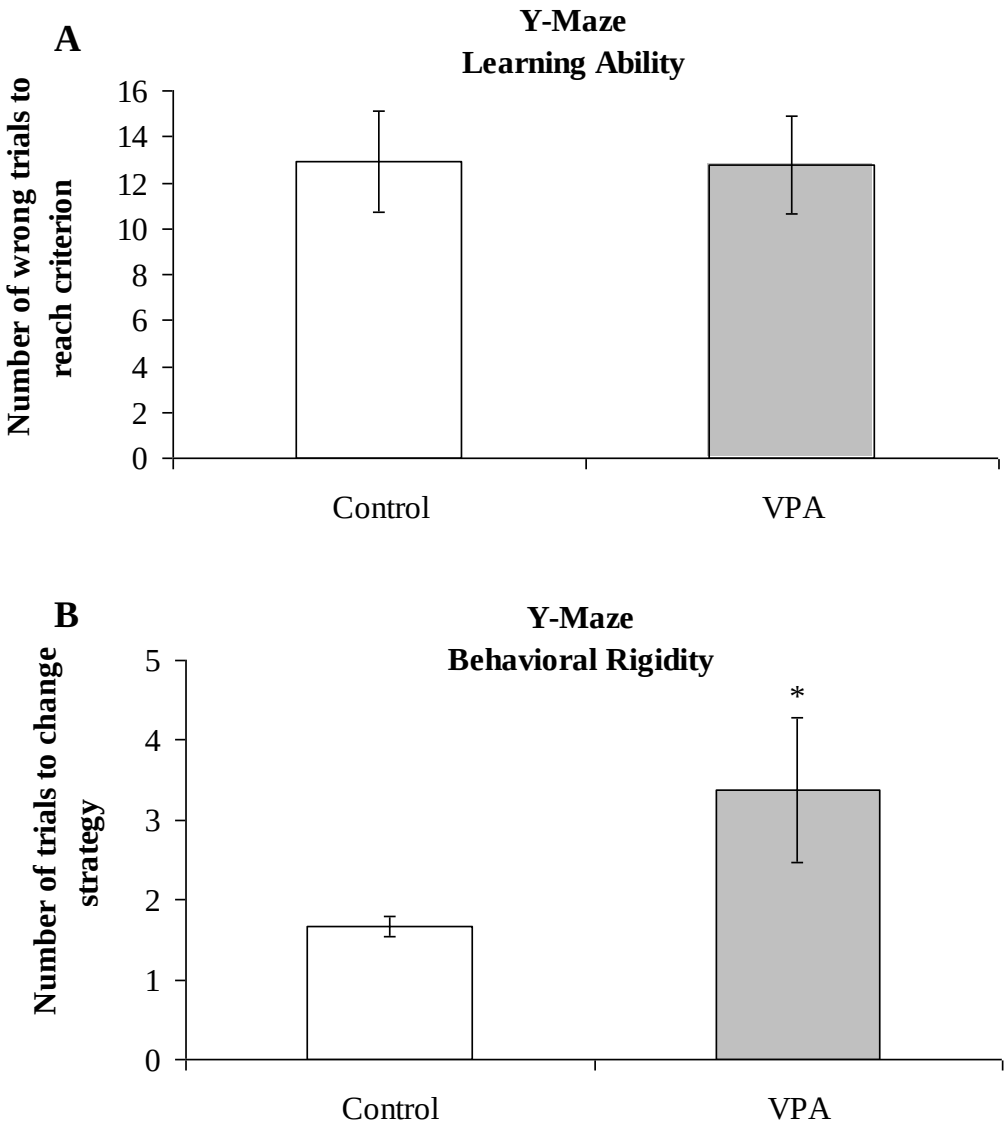

A
Y-Maze
Learning Ability
Number of wrong trials to reach criterion
16
14
12
10
8
6
4
2
0
Control
VPA
B
Y-Maze
Behavioral Rigidity
Number of trials to change strategy
5
4
3
2
1
0
*
Control
VPA

Figure 3

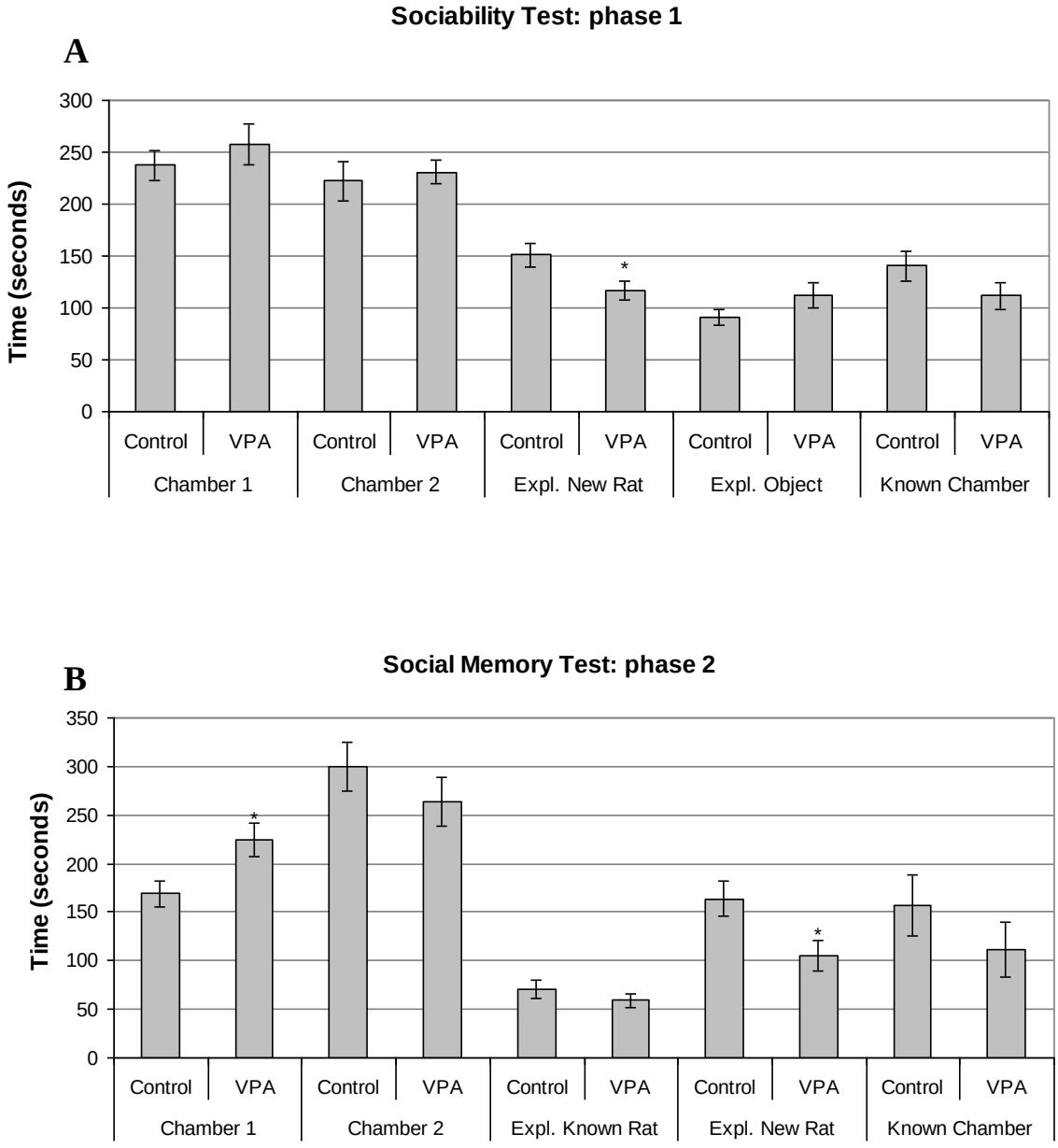

Sociability Test: phase 1
A
Time (seconds)
300
250
200
150
100
50
0
*
Control
VPA
Chamber 1
Chamber 2
Expl. New Rat
Expl. Object
Known Chamber
Social Memory Test: phase 2
B
Time (seconds)
350
300
250
200
150
100
50
0
*
*
Control
VPA
Chamber 1
Chamber 2
Expl. Known Rat
Expl. New Rat
Known Chamber

Figure 4

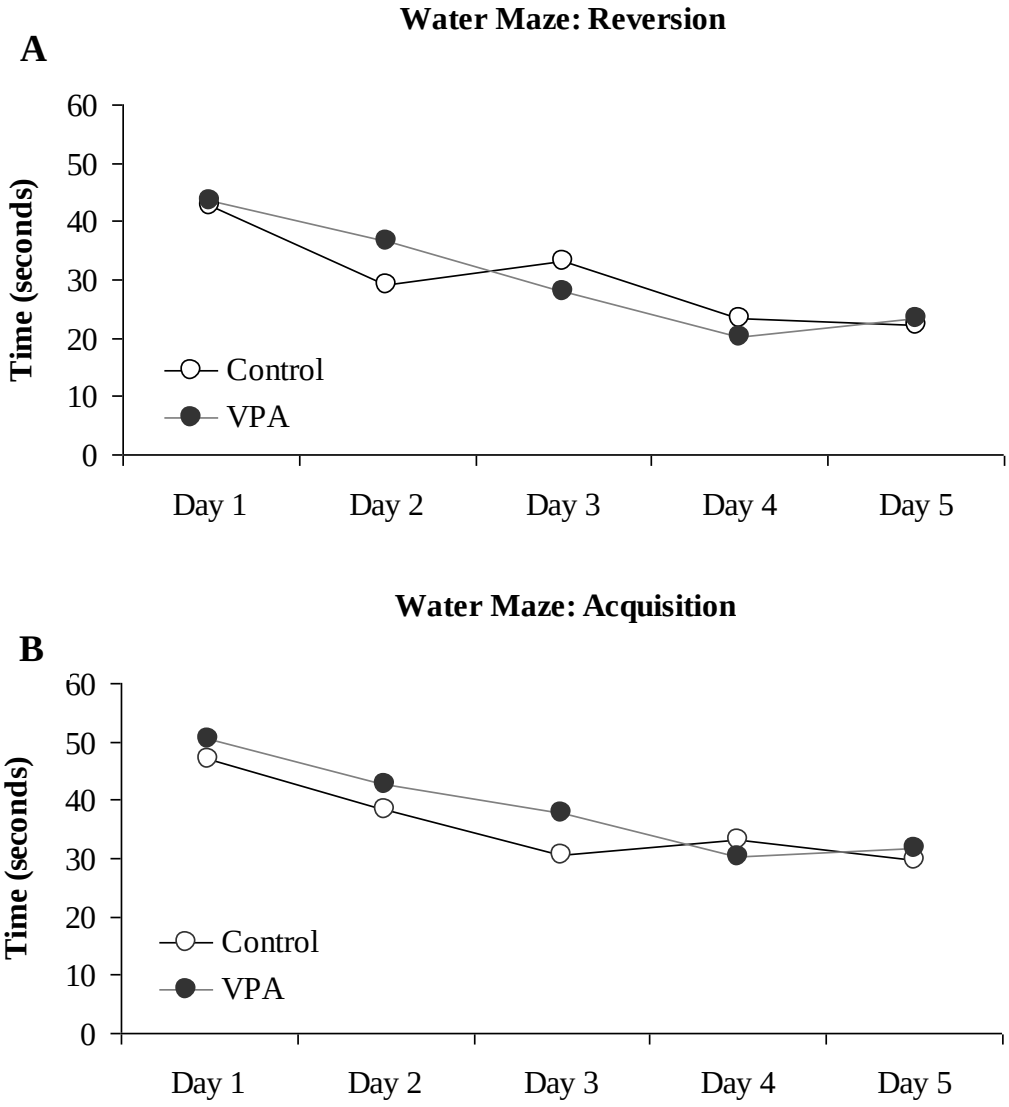

A
Water Maze: Reversion
Time (seconds)
60
50
40
30
20
10
0
Control
VPA
Day 1
Day 2
Day 3
Day 4
Day 5
B
Water Maze: Acquisition
Time (seconds)
60
50
40
30
20
10
0
Control
VPA
Day 1
Day 2
Day 3
Day 4
Day 5

Figure 5

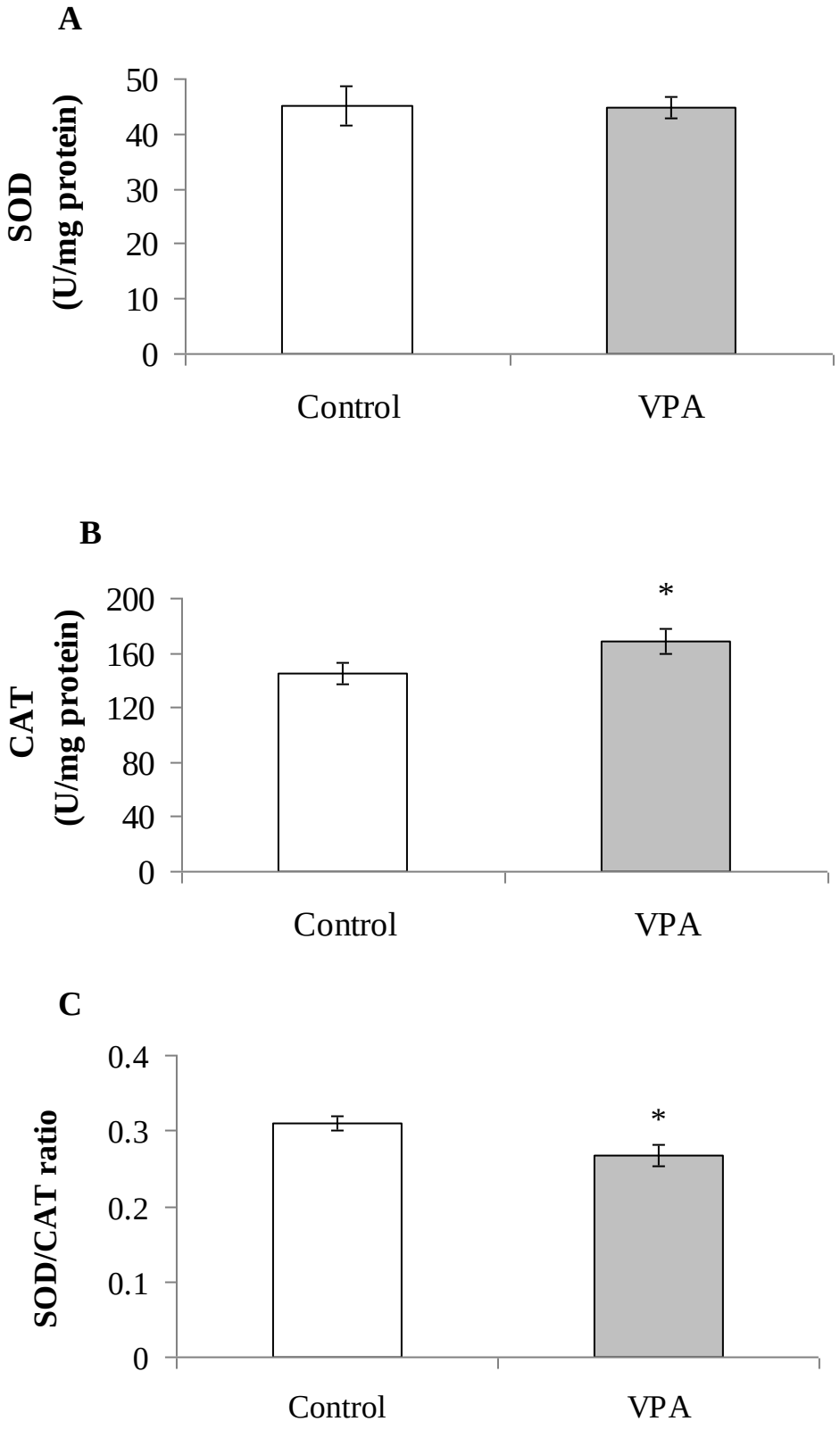
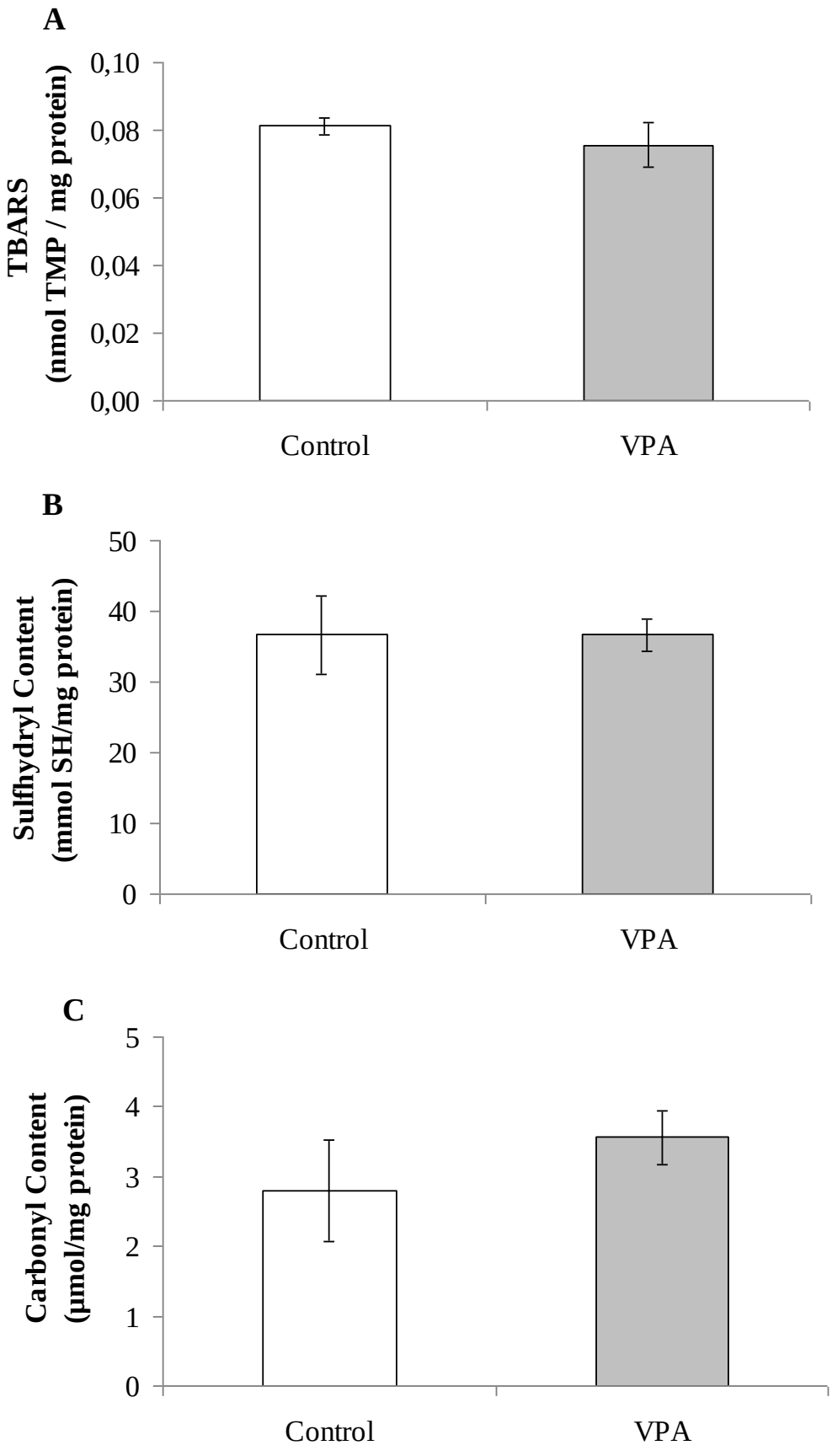

A
SOD (U/mg protein)
50
40
30
20
10
0
Control
VPA
B
CAT (U/mg protein)
200
160
120
80
40
0
*
Control
VPA
C
SOD/CAT ratio
0.4
0.3
0.2
0.1
0
*
Control
VPA

Figure 6


A
TBARS
(nmol TMP / mg protein)
0,10
0,08
0,06
0,04
0,02
0,00
Control
VPA
B
Sulfhydryl Content
(mmol SH/mg protein)
50
40
30
20
10
0
Control
VPA
C
Carbonyl Content
(µmol/mg protein)
5
4
3
2
1
0
Control
VPA

4. DISCUSSÃO E CONSIDERAÇÕES FINAIS

O principal objetivo do nosso trabalho foi estabelecer o modelo de autismo em ratos induzido por exposição pré-natal ao VPA, verificando através de mudanças em padrões comportamentais, o desenvolvimento de características autistas. Além disso, observarmos se as alterações comportamentais são decorrentes somente de efeito sobre o SNC ou se também tem efeito metabólico sistêmico, através da análise de alguns elementos importantes do metabolismo de roedores.

O modelo animal de autismo induzido por VPA apresenta diversas características comportamentais observadas em portadores de autismo. Entre essas se destacam: comportamentos repetitivos e estereotipados, diminuição na quantidade de comportamentos sociais (Schneider et al. 2006), dificuldades para iniciar um contato social (Dufour-Rainfray *et al.* 2010), e anomalias no ciclo circadiano (Tsujino et al. 2007).

O modelo descrito por Schneider (Schneider et al. 2006) demonstra que o enriquecimento ambiental reverte uma série de comportamentos anômalos como latência no *Tail Flick*, diminuição na quantidade de comportamentos repetitivos e estereotipados, além de normalizar a exploração social.

No presente, avaliamos memória social (através do teste do labirinto de três câmaras em duas fases), rigidez comportamental (pela reversão no labirinto em Y e no MWM) e parâmetros bioquímicos. No aparato em três câmaras, executado com os ratos jovens, o tempo gasto explorando o rato "estranho" pelo grupo VPA foi significativamente menor, indicando um comportamento social aberrante. Essa é uma disfunção social apresentada

pelos ratos expostos pré-natalmente ao VPA, a qual também é comum aos portadores de autismo (DiCicco-Bloom et al. 2006, Crawley 2007). Ainda no labirinto três câmaras, os ratos controle apresentaram uma preferência condicionada pelo seu semelhante, passando mais tempo na câmara que continha o rato “estranho” do que na câmara que continha o objeto ($p<0,05$). Esse comportamento social típico de roedores não foi evidenciado pelo grupo VPA.

No labirinto em Y, uma tarefa de discriminação espacial, não foi constatada nenhuma diferença entre os grupos para atingir o critério, desta forma, podemos sugerir que os ratos VPA não apresentam retardo de aprendizado, ao menos nesta tarefa. Entretanto, essa é uma característica freqüentemente associada ao autismo (Casanova 2007). Uma vez que eles haviam aprendido a tarefa normalmente, quando submetidos a uma situação em que deveriam alterar sua estratégia, escolhendo o braço contrário, os ratos VPA apresentaram uma latência maior até realizarem uma tentativa diferente. Este resultado tem grande importância para o estabelecimento deste modelo animal para o estudo do autismo, uma vez que pode ser correlacionado com a rigidez comportamental e as dificuldades de adaptação a uma nova rotina, apresentadas pelos portadores de autismo.

Quando adultos, foram submetidos aos testes de duas fases no aparato de três câmaras e ao labirinto Aquático de Morris (aquisição e reversão). Na tarefa social pode-se ter uma boa perspectiva das alterações comportamentais apresentadas pelos ratos expostos ao VPA durante a gestação. Na primeira fase do teste em três câmaras, novamente o grupo VPA permaneceu menos

tempo interagindo socialmente com o rato “estranho” do que o grupo controle. Na segunda fase do teste onde a preferência por novidade social foi mensurada, os ratos VPA mostraram menor interesse em fazer um novo contato social. Isso porque passaram menos tempo com o novo “estranho” do que os ratos controle. Além disso, o tempo utilizado para o contato social com o primeiro “estranho” na segunda fase foi semelhante em ambos os grupos. Isso significa que os ratos VPA provavelmente tenham reconhecimento social normal porque apresentaram o mesmo grau de interesse que os controles pelo primeiro “estranho” na segunda fase, e, além disso, por explorarem o novo estranho mais do que o primeiro.

O grupo VPA também passou mais tempo na câmara um na segunda fase, este fato pode ser uma conseqüência que os ratos VPA estariam mais confortáveis em uma câmara nas quais eles não estariam submetidos a um novo contato social.

O aprendizado no Labirinto Aquático de Morris apresentado pelos ratos VPA foi semelhante ao dos ratos controle, evidenciando mais uma vez a capacidade normal de aprendizado deste grupo. Além disso, quando submetidos ao Labirinto Aquático de Morris Reverso, aprenderam a nova tarefa tão rapidamente quanto os ratos controle e não mostraram a mesma dificuldade de adaptação apresentada anteriormente, no Labirinto em Y.

De acordo com os dados acima, e uma vez que os ratos foram sistemicamente submetidos a um teratogênico (VPA), tentamos elucidar se estes comportamentos aberrantes advêm seletivamente de um dano encefálico ou, pelo menos, em parte do comprometimento de outras vias metabólicas

(Ingram et al. 2000, Binkerd *et al.* 1988, Christianson *et al.* 1994). A partir disso, parece próprio supor que o fígado, um órgão que desempenha uma função primordial na manutenção fisiológica do organismo, é um lugar importante para procurar possíveis danos ou alterações metabólicas induzidas pela exposição a um teratogênico. Para analisar a integridade hepática, quantificamos no soro destes animais, a atividade das enzimas citoplasmáticas e marcadoras de hepatotoxicidade, AST/GOT e ALT/GPT. A atividade não diferiu entre os grupos, evidenciando uma consequente ausência de dano. Além disso, analisamos danos oxidativos que poderiam comprometer a integridade celular sem causar uma ruptura da membrana citoplasmática do hepatócito. Porém, nenhum dano foi observado em proteínas (pela quantidade de radicais sulfidril e grupos carbonil) ou lipídios (TBARS). As atividades das enzimas SOD e catalase, também foram avaliadas, sendo que a atividade catalase estava aumentada nos ratos VPA. Entretanto, esta atividade aumentada de modo algum representou um comprometimento das funções celulares, uma vez que não foi encontrado dano oxidativo.

5. CONCLUSÕES

Conclui-se com este trabalho que o modelo animal de autismo estabelecido através da exposição pré-natal ao VPA, além das características já descritas na literatura, apresenta rigidez comportamental durante a adolescência. Já o comportamento social demonstrou-se aberrante na adolescência, e na idade adulta, sendo que nesta também se observa uma memória social íntegra e uma busca reduzida por aproximação social por parte dos ratos VPA. Além disso, conclui-se que a exposição pré-natal ao VPA não afetou a integridade hepática e, pelos parâmetros estudados, não provocou dano em lipídeos e em proteínas, confirmando que as diferenças comportamentais encontradas não derivam de algum comprometimento hepático. Desta forma, pode se concluir que o modelo estabelecido apresenta características importantes de correlação com o espectro autista, tornando-se uma estratégia metodológica fundamental para estudos posteriores relacionados com autismo.

6. PERSPECTIVAS

- Avaliar alterações na expressão gênica e protéica dos receptores NMDA, AMPA e GABA(A) em diferentes regiões encefálicas, incluindo amígdala e hipocampo.

- Avaliar por microscopia, a densidade e morfologia de espinhos dendríticos nos diferentes núcleos da amígdala, bem como a distribuição destes receptores.

- Avaliar o efeito de antipsicóticos atípicos sobre comportamento agressivo, investigando parâmetros de sinalização neuroglial.

- Investigar a influência de terapias cognitivo-sociais, tais como musicoterapia e enriquecimento ambiental sobre parâmetros comportamentais e bioquímicos.

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