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# Combined prenatal and postnatal butyl paraben exposure produces autism-like symptoms in offspring: Comparison with valproic acid autistic model

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ABSTRACT

The aim of this work is to evaluate the impact of butyl paraben (BP) in brain of the pups developed for mothers administered BP from early pregnancy till weaning and its effect on studying the behavior, brain neurotransmitters and brain derived neurotrophic factor BDNF via comparing the results with valproic acid (VA) autistic-rat model preparing by a single oral injection dose of VA (800 mg/kg b.wt) at the 12.5 days of gestation. Butyl paraben was orally and subcutaneously administered (200 mg/kg b.wt) to pregnant rats from gestation day 1 to lactation day 21. The offspring male rats were subjected at the last 3 days of lactation to Morris water maze and three chamber sociability test then decapitated and the brain was excised and dissected to the cortex, hippocampus, cerebellum, midbrain and pons for the determination of norepinephrine, dopamine and serotonin (NE, DA and 5-HT) and cortex amino acids and whole brain BDNF. The results showed similar social and learning and memory behavioral deficits in VA rat model and the butyl paraben offspring in comparison with the controls. Also, some similar alterations were observed in monoamine content, amino acids and BDNF factor in the autistic-like model and butyl paraben offspring in comparison with the controls. The alterations were recorded notably in hippocampus and pons NE, midbrain DA, hippocampus and midbrain 5-HT, and frontal cortex GABA and asparagine. These data suggest that prenatal exposure to butyl paraben induced neuro-developmental disorders similar to some of the neurodevelopmental disorders observed in the VA model of autism.



## 1. Introduction

Autism is one of the highest growing developmental disorders, characterized by inhibited mutual social interactions, communication deficits and marked inflexibility to environmental changes, which makes therapy extremely difficult (Markram et al., 2007). Autism spectrum disorders (ASD) are a group of neurodevelopmental disorders involving autistic disorder, Asperger disorder and pervasive developmental disorder (Kogan et al., 2009). In fact, ASD is not a disease but rather a syndrome that is characterized by a multifactorial type of inheritance. In some cases, it is one of the symptoms of monogenic or chromosomal pathology, and can also be a symptom of inherited metabolic disorders (Muhle et al., 2004). The International Classification of Diseases distinguishes between autism and ASD, which are subdivided into syndromic autism and non-syndromic or idiopathic autism (Persico and Lintas, 2009). There is also a hypothetic classification of ASD and autism based on the difference in phenotypical features (Miles et al., 2008). Autism is considered one of the ASD group disorders and is clinically characterized by impairments in three major domains: 1) social interaction; 2) language, communication and imaginative play; and 3) range of interests and activities (Gadia et al., 2004; Rapin and Tuchman, 2008). Additionally, males are affected by autism four times more than females by the ratio 4.3:1 (Fombonne, 2005, 2007). There are no existing statistics on the prevalence of ASD in Egypt (Fahmy et al., 2013). Autism is considered a rare disorder before the past 2 decades, but the recent epidemiological studies on American and European populations demonstrate a sharp increase in its prevalence (Chakrabarti and Fombonne, 2001; Fombonne, 2003). The prevalence of ASD is increased from 1:10,000 children in 1970s (Kawamura et al., 2008) to 116.1:10,000 children in UK (Baird et al., 2006). ASD is influenced by a grouping of various genetic, environmental and immunological vulnerabilities to oxidative stress agents (Gargus and Imtiaz, 2008).

Parabens are preservatives used in a wide range of cosmetic products, including products for children, and some are permitted in foods, toiletries, and pharmaceuticals (Oishi, 2001). However, there is a concern for endocrine disrupting effects (Boberg et al., 2010). Studies in young male rats have shown adverse action on sperm production and testosterone levels following oral exposure to parabens with longer side chains, i.e. butyl- and propylparaben (Oishi, 2001, 2002).

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Furthermore, parabens are known to be estrogenic in vitro and in uterotrophic assays in vivo, and estrogenicity appears to increase with side chain length (Darbre and Harvey, 2008).

Butyl paraben is an ester of para-hydroxy benzoic acid which has a chemical structure like alkyl phenols, It is one of the most potent endocrine disruptors (Oishi, 2001). It is involved in the production of reactive oxygen species ROS in HepG2 cell cultures (Khanal et al., 2012). It has been demonstrated that a number of environmental pollutants are involved in the prevalence of ASD such as heavy metals, organohalogens, pesticides and plasticizers (Schwartzer et al., 2012).

Serotonin, GABA, glutamate, norepinephrine and dopamine have been investigated in relation to the pathology of autism (Wegiel et al., 2010; Choudhury et al., 2012; Mori et al., 2012). Imbalance in the serotonin system has been found in autistic population and includes hyperserotonemia, which can harm the developing brain (Whitaker-Azmitia, 2005). Alterations in serotonergic innervations and tone are evident in autism (Boylan et al., 2007). Based on the amount of affected brain structures and neurotransmitter pathways typically affected in autism and behavior related to social deficit (Olexova et al., 2012). Glutamate, the main excitatory neurotransmitter actively participates in different neuro-developmental processes through complex regulatory events. Excitatory neurotransmitter signaling via glutamate receptors modulates cognitive functions such as memory and learning, which are usually impaired in ASD (Choudhury et al., 2012). Increasing evidence suggests that an imbalance between excitatory glutamate and inhibitory gamma-amino-butyric-acid (GABA) neurotransmission may form a final common pathway in ASD. In particular, abnormality in GABA transmission, leading to brain hyper-excitability, have been implicated in the patho-physiology symptoms of ASD (Rubenstein and Merzenich, 2003; Yizhar et al., 2011).

The most abundant neurotrophin in the brain is the brain-derived neurotrophic factor (BDNF). BDNF is widely circulated in the cortex, hippocampus, basal forebrain, striatum, hypothalamus, brain stem and cerebellum. BDNF is most abundant in the hippocampus formation, amygdaloid complex and prefrontal cortex (Murer et al., 2001; Pillai, 2008). It is also expressed in neurons, astrocytes, microglia and endothelial cells, and is localized to the soma, dendrites and fibers (Pillai, 2008). BDNF transcripts increase throughout brain development (Friedman et al., 1991) and its expression does not appear to decline with age, indicating an essential role for maintaining the adult CNS (Narisawa-Saito and Nawa, 1996; Katoh-Semba et al., 1997). It serves an important role during brain development and in synaptic plasticity (Hong et al., 2012). BDNF is implicated in cognitive function and personality development as well as the pathogenesis of various psychiatric diseases, including mood disorders, schizophrenia and autism (Hashimoto et al., 2004; Angelucci et al., 2005; Berton and Nestler, 2006; Hashimoto et al., 2006).

The aim of this work is to evaluate the neuro-development impairment induced by prenatal and postnatal exposure to butyl paraben by comparing the behavior, whole brain BDNF and neurotransmitters level in different brain areas with VA autistic-like model and control trying to answer the question that is the butyl paraben in normal dose (for mothers “less than 0.19% in human”) could induce autism-like symptoms in the offspring rats?.

## 2. Material and methods

### 2.1. Experimental animals

Eighty female albino rats weighing 200 ± 20.5 g and 30 male rats weighing 250 ± 22.1 g were obtained from the animal house of National Organization for Drug Control and Research (NODCAR). The female rats were mated overnight and vaginal lavage was used to test for the presence of sperm. The first day of gestation was designated as the day when the smear was sperm-positive (Dendrinos et al., 2011). The rats were housed in plastic cages, each cage contained four pregnant female rats. Animals were kept under controlled temperature of 25 ± 2 °C and 12 hour light/12 hour dark cycle throughout the experiment. A commercial rodent pelleted diet was used during the experiment. The animals were allowed to adapt to the laboratory conditions for two weeks before the beginning of the experiment. Food and water were available ad libitum. The experimental protocols and procedures were approved by the state authorities and followed Egyptian rules for animal protection.

### 2.2. Drugs

Butyl paraben BP ($C_{11}H_{14}O_3$) was supplied as a pure white powder (assay ≥ 99.0%) with a code no (54680-50g-F) from Sigma Aldrich. St Louis, MO, USA. Valproic acid sodium salt ($C_8H_{15}NaO_2$) was supplied from Sigma-Aldrich St. Louis, MO, USA cat no1069-66-5 (assay ≥ 98%). Tween 80 was used as vehicle.

### 2.3. Experimental design

The 60 male offspring rats were divided into five main groups; each of 12 rats as follows: 1 — the control group (CSc), which contains the offspring of pregnant mothers received 0.25 ml/100 g b.wt. of 1 ml/100 ml tween 80/day subcutaneously; 2 — the control group (COi), which contains the offspring of pregnant mothers received 0.25 ml/100 g b.wt. of 1 ml/100 ml tween 80/day orally; 3 — the autistic-like group (VA) which contains the offspring of the pregnant mothers treated as the previous group plus 800 mg valproic acid sodium salt/kg orally as one dose on the 12.5 gestational day (Kuwagata et al., 2009; Kim et al, 2011); 4 — offspring of the butyl paraben subcutaneous administration group (BPSc) which the pregnant mothers received 200 mg BP/kg/day (BP, SCCS, 2010); and 5 — offspring of the butyl paraben oral administration group (BPOi), which the pregnant mothers received 200 mg BP/kg/day (Aubert et al., 2012). All treatments were administered daily for six consecutive weeks from the first gestation day to the 21 lactation day. At 19th lactation day, the test of the Morris water maze began for two consecutive days before decapitation. The three box chamber test was performed at the 21st day of lactation. At 22nd day of lactation, the offspring males were decapitated and the brain was excised and dissected immediately on ice.

### 2.4. Three-chamber sociability test

The social test was performed in a three chambered apparatus as described previously in a mice task (Nadler et al., 2004), without modifications required to perform it in rats because the rats were small about 40 g at age 21 days. It is a Plexiglass box with partitions separating the box into three chambers with dimensions (length/width/height in cm) 60/40/30. The openings between compartments allowed free exploration to the different chambers. Time spent in each chamber, as well as the time spent exploring the stranger rat or an object in the chamber, was analyzed. The object was an empty identical cage used to enclose the stranger rat. Chambers were cleaned with 70% ethanol and water between tests. Animals used as “strangers” were males with the same age“21 days” and no previous contact with the test rats. For the sociability test, rats were allowed to expend 10 min in the central chamber, and then the stranger rat was introduced into one of the side chambers. The experiment was performed for up to 10 min, with the stranger rat and an object on each side. 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. The number of entries was recorded when the four limbs of the rat passed the gate of the chamber.

### 2.5. Morris water maze task

The Morris water maze is a circular pool with a featureless inner black surface and a diameter and height of 160 and 60 cm, respectively. The pool was filled to a depth of 45 cm with water. The pool was placed