American Medical Journal 4 (2): 190-196, 2013
ISSN: 1949-0070

doi:10.3844/amjsp.2013.190.196 Published Online 4 (2) 2013 (http://www.thescipub.com/amj.toc)

Effect of Bisphenol a on Passive Avoidance Performance in Male Rats

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Received 2012-05-08; Revised 2012-06-17; Accepted 2013-12-10

ABSTRACT

Bisphenol A (BPA) is used to manufacture polycarbonate plastic products in resins lining metal cans, in dental sealants and in blends with other types of plastic products. In sharp contrast, there are many published studies that BPA has a wide range of significant adverse effects including structural and neurochemical changes throughout the brain associated with behavioral changes, such as hyperactivity, learning deficits and increased aggression in both males and females. So the aim of the present study was to investigate effect of BPA on learning and memory in passive avoidance learning model. Thirty six male Sprague Dawley rats weighing 180-260 g were used. The animals were divided into six groups: 1- control group (without any treatment) 2-sham group (received sesame oil the same volume as experimental, n = 6); 3-5 experimental (received BPA 5, 50, 100 and 150 mg kg^{-1} day, n = 24). BPA was administrated by oral intake by gavage for 15 days. Learning and memory were performed by shuttle-box. Data was analyzed by Kruskal-Wallis nonparametric test. The level of significance was considered $P<0.05$. Our data showed that BPA does not have a significant difference in time spent in light in doses of 5, 50 mg kg^{-1} day in learning and memory. BPA with dose of 100 and 150 mg kg^{-1} day showed a significant decrease in time spent in light relative to the control and sham in learning and memory. According to our results, BPA impaired learning and avoidance memory with high doses in the passive avoidance learning task.

Keywords: BPA, Memory, Shuttle-Box, Rats

1. INTRODUCTION

The synthetic xenoestrogens, Bisphenol A (BPA) is one of the highest volume chemicals and its production capacity in 2003 in USA was 2.2 million tonnes (over 6.4 billion pounds), with a 6-10% growth in demand expected per year (Slayton *et al*., 2006). BPA, an industrial chemical used in the manufacturing of epoxy and polycarbonated resins, has gained attention in recent years because of its potentially determined effects on human health (Diamanti-Kandarakis *et al*., 2009). Heat and either acidic or basic environments accelerate hydrolysis of the ester bond linking BPA monomers which leads to the release of BPA and the potential for human and environmental exposure (Coors *et al*., 2003; Kawagoshi *et al*., 2003). BPA leaches into foods from plastic wrappings, baby bottles and coating inside of food cans (Zhang *et al*., 2010). In the central nervous system, gonadal steroid hormones not only influence neurodevelopment and sexual differentiation, but also have a so-called "activation effect", an effect involving the modulation of function and activity in the mature brain (Breedlove, 1994). It is well documented that both estrogens and androgens significantly influence cognitive performance in human beings as well as in laboratory animals (Edinger and Frye, 2007; Mukai *et al*., 2006). BPA is one of the well-known "environmental endocrine disrupters" that is known to have mixed

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estrogen agonist/antagonist properties (Welshons *et al*., 2006). It can interfere with hormone synthesis and clearance, as well as alter hormone receptor expression and gene activity in target tissues (MacLusky *et al*., 2005). Kim *et al*. (2004) reported that BPA has very weak estrogenic activity and can potentially accumulate in neuroendocrine target organs such as the gonads and brain (Kim *et al*., 2004). There is concern that the exposure of embryo and/or infants to BPA may lead to neurological and behavioral disorders (Braun *et al*., 2009). In the embryonic mouse brain BPA changes the timing of cell birth in the cortex (Nakamura *et al*., 2007). In the neonatal rat brain BPA has estrogenic actions and increases dendritic lengths in cerebellar purkinje cells (Shikimi *et al*., 2004). The synaptic density in hypothalamic neurons of rat was significantly increased by administration of 100 nM BPA for 7 days (Yokosuka *et al*., 2008). In contrast, BPA can block the acts of estrogens on the formation of spine synapses in adult primates (Leranth *et al*., 2008). Co-administration of BPA and testosterone in castrated rats revealed BPA prevented both hippocampus and prefrontal cortex synaptogenic responses to testosterone, confirming that BPA acts as an anti-androgen, therefore BPA administration results in a severe loss of spine synapses in both the hippocampus and prefrontal cortex; there are no differences between sexes in the response to BPA in adult rats, at least in the spine synapse remodeling (Hajszan and Leranth, 2010). BPA possibly affected more than one pathway of memory modulation. Xu *et al*. (2010) reported that prenatal BPA exposure significantly impaired spatial memory and passive avoidance memory during postnatal developing stage and the adulthood of male offspring (Xu *et al*., 2010). Miyagawa *et al*. (2007) suggest that prenatal and neonatal exposure BPA have no direct effect on motor skill learning. But chronic exposure with low and high doses of BPA induced the memory impairment in passive avoidance task (Miyagawa *et al*., 2007). On the other hand Xu *et al*. (2010) showed that acute exposure to BPA enhanced memory in passive avoidance task (Goncalves *et al*., 2010). Therefore, the aim of the present ultstudy was to investigate the effect of BPA on passive avoidance learning in adult male rats.

2. MATERIALS AND METHODS

All the procedures involving animal subjects were reviewed and approved by the Institutional Research Ethics Committee of the School of Veterinary Medicine of Shiraz University.

Thirty six male Sprague Dawley rats weighing 200-220 g were used. Food and water were made available ad libitum. The rats were housed under a 12 h light/dark (light on at 6 a.m.) and controlled temperature (20±4°C) condition.

The rats were randomly divided (random assignment) into 6 equal groups (n = 6 each) 1- control group (without any treatment) 2-sham group (received sesame oil the same volume as experimental, n = 6); 3-5 experimental (received BPA 5, 50, 100 and 150 mg kg^{-1} day, n = 24) and administrated with BPA (purchased from sigma-Aldrich Co) dissolved in sesame oil (5, 50,100 and 150 mg kg^{-1} day) or only sesame oil as a vehicle by oral gavages[21] for 15 days. A two-way shuttle-box (made by Aryoazma Co) with acrylic walls and steel floor bars was used. The box, 44×20×19 cm, was bisected by a vertical partition with an opening in the middle that allows the animal to move freely from one compartment to other, including light and dark compartments. In the light compartment the animal was safe while in the dark compartment it received a foot shock of 0.6 mA for one sec; with a latent period of one sec.

2.1. Procedure

On the first day, all animals were individually subjected to 2 min of adaptation to the shuttle box, in which the rat could explore the light compartment and move about freely; at this stage since the rat likes dark compartment, if the rat did not move to dark compartment after 120 sec it was removed from study. This adaptation was repeated 30 min later. On the second day, the rats were placed in the light compartment box and one sec after entering to the dark compartment received a 0.6 mA foot shock for one sec. On the third day, the procedure was similar to the second day; the third day considered as learning. On the fourth day, as memory consolidation, the procedure was like the learning days without foot shock. On the fifth day, as memory retention, the procedure was similar to the fourth day. The rats were considered as completely learned, if they did not move to dark compartment after 120 sec during third, fourth and fifth session of experiments.

2.2. Data Analysis

Number of rats was determined according to a pilot study using the following formula: $n = [(Z\alpha+Z\beta)SD/\text{mean difference}]\times 2$ using $Z\alpha = 1.96$, $Z\beta = 0.84$, SD = 0.06 and mean difference of 0.07 yielded a sample size of 4.62 for each group, therefore a sample of five rats were included in each group. Data, presented as mean±SEM, were analyzed by Statistical Package for Social Sciences (SPSS, version 18). Data were analyzed separately for each group with Kruskal-Wallis nonparametric test. In case of significant results with Kruskal-Wallis test, pairwise

comparisons were made using mann-whitney test. A P value of <0.05 was considered statistically significant.

3. RESULTS

BPA administrated to all animal during 15 days in doses of 5, 50, 100 and 150 mg kg^{-1} day^{-1}. On the learning session BPA in doses of 5, 50 mg kg^{-1} day^{-1} showed no significant (P = 0.657) difference in time spent in the light compartment compared to the control and sham group, but BPA in doses of 100 and 150 mg kg^{-1} day^{-1} significantly (P = 0.0005) decreased time spent in the light compartment compared to the control and sham group (**Fig. 1**).

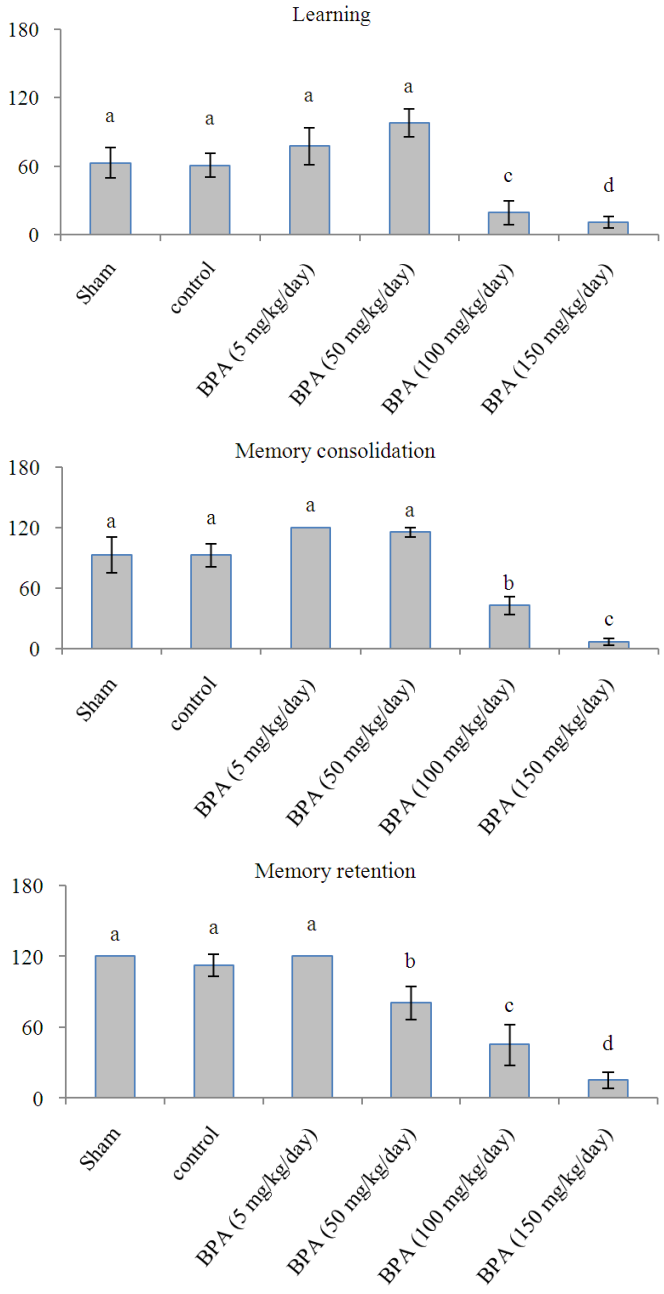

Fig. 1. Mean latencies (±SEM) of each group of rat during learning, memory consolidation and memory retention

On the memory consolidation day BPA in doses of 5, 50 and 100 mg kg^{-1} day^{-1} showed no significant (P = 0.758) difference in time spent in the light compartment compared to the sham group but in dose 150 mg kg^{-1} day^{-1} significantly (P = 0.001) decreased time spent in the light compartment compared to the control and sham group (**Fig. 1**). On the memory retention day BPA at doses 50,100 and 150 mg kg^{-1} day^{-1} significantly (P = 0.0003) decreased time spent in the light compartment compared to the control and sham group (**Fig. 1**). These results showed that BPA at a dose of 5 mg kg^{-1} day^{-1} was not sufficient dose to change in learning and memory, but in dose of 50 mg kg^{-1} day only impaired memory retention; on the other hand in doses 100 and 150 mg kg^{-1} day impaired learning and memory compared to the control and sham group.

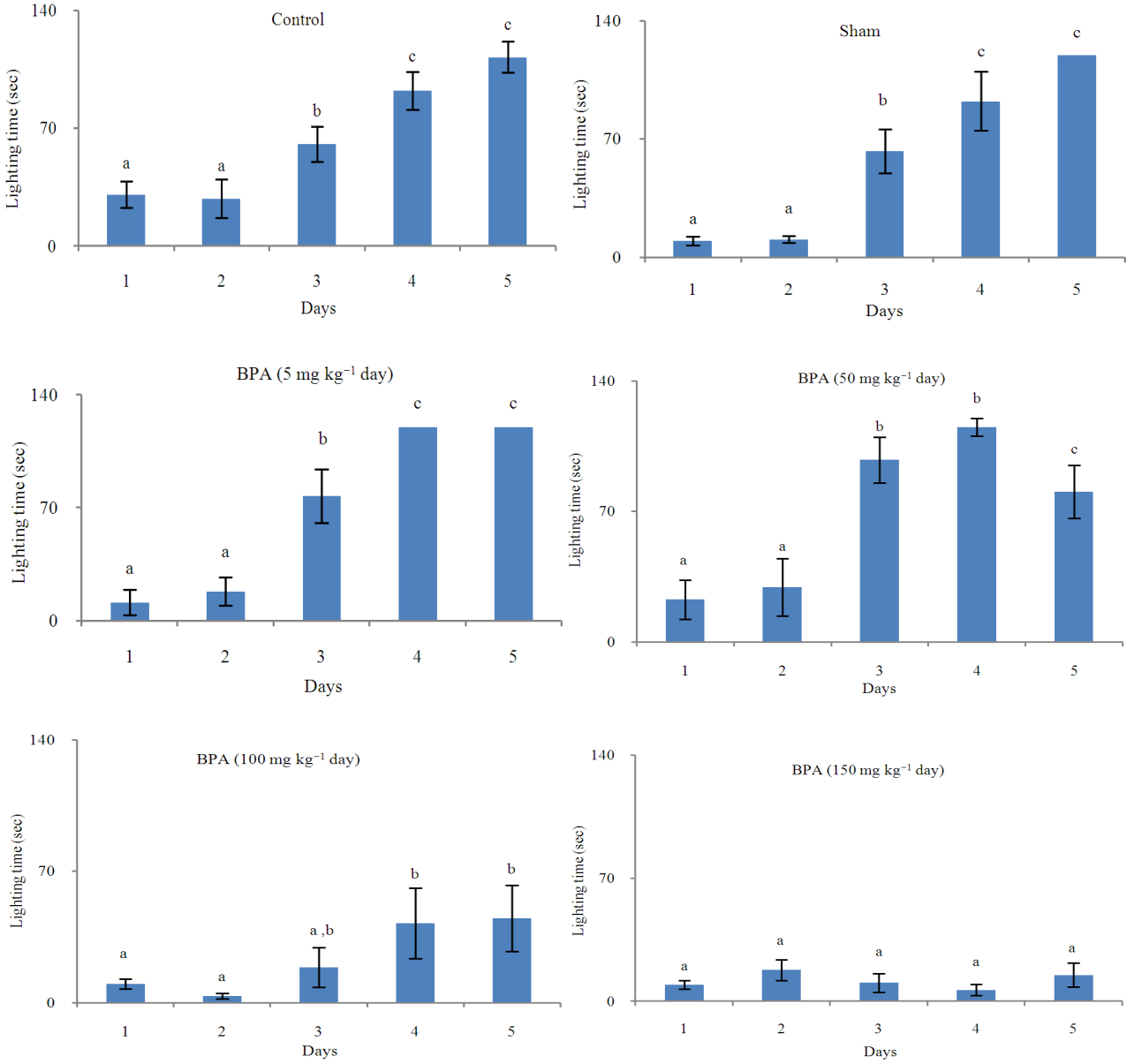

Fig. 2. Mean latencies (±SEM) of each group of rat during day1 (an introduction to shuttle box); day2 (Initial latency); day3 (learning); day4 (memory consolidation) and day5 (memory retention)

For investigation of learning and memory processing in each groups during 5 days; in the control, sham and BPA 5 and 50 mg kg^{-1} day groups there were a significant (P = 0.0001) difference during the 5 days of experiment, so it mean that learning and memory was normal (**Fig. 2**). Administration of BPA at doses 100 and 150 mg kg^{-1} day did not induced any significant changes in the time spent in the light compartment during 5 day of test period; so in higher doses BPA disrupt formation of learning and memory (**Fig. 2**).

4. DISCUSSION

In the present study shock intensity was 0.6 mA (for one sec). Everss and Parra (1998) showed that shock intensity 0.6 mA was high enough to produce inhibitory avoidance conditioning.

In the present study, BPA in doses 5 and 50 mg kg^{-1} day had no effects on learning and memory consolidation and BPA only in dose 50 mg kg^{-1} day did it decrease retention of memory. On the other hand, BPA in 100 and 150 mg kg^{-1} day has impaired learning and memory. The perturbation of play and maze learning behaviors was reported in both female and male rodents after developmental BPA administration. Goncalves *et al*. (2010) reported that BPA administration during gestation resulted in no appearant change in memory and behavioral tasks. Nevertheless, BPA administration in the lactation caused important determental effects on both sexes (Goncalves *et al*., 2010). The results may be the basis for the ability of BPA to interfere with spine synapse formation in the prefrontal cortex and hippocampus to have some clinical implications (Carr *et al*., 2003). Zhou *et al*. (2011) showed that the $GABA_A$ receptor agonist could block the induction of NMDAR-dependent LTP in BPA-treated rats. However, GABAergic dysfunction may not be the only mechanism to cause the facilitated LTP induction in BPA-rats, because the blockade of $GABA_A$ receptor alone in control rats was insufficient to facilitate the LTP induction (Zhou *et al*., 2011).

The findings of Xu *et al*. (2010) showed that prenatal BPA administration significantly impaired spatial and passive avoidance memories during the postnatal developing stage and the adulthood of male offspring. Poimenova *et al*. (2010) reported that a long term prenatal administration of a safe BPA dose can impair spatial recognition memory for a Y-maze task in mid-adolescent rats of both sexes (Poimenova *et al*., 2010). In spatial memory paradigm (water-maze) with preexisting male superiority, postnatal administration of rats to low BPA concentration abolished the male dominance in the acquisition of the task, while a higher BPA dose was required to impair the performance of adolescent female offspring (Carr *et al*., 2003). In a study conducted only in male mice, BPA impaired memory for a step-through passive avoidance and significantly reduced choline acetyltranferase-like immunoreactivity in the hippocampus (Miyagawa *et al*., 2007). Leranth *et al*., (2004) demonstrated that gonadal steroid hormones are responsible for maintaining physiological levels of spine synapses in the prefrontal cortex and the hippocampus of non human primates. Changes in synaptic connections are essential for the development and function of the nervous system. BPA appears to strongly interfere with these mechanisms, as continuous administration of a lower dose, 50 μg kg^{-1} daily completely abolishes gonadal steroid hormone-induced spine synapse growth (Leranth *et al*., 2004). So, it is conceivable that BPA may have a widespread influence on the structure and function of the brain, as remodeling of prefrontal and hippocampal spine synapses plays a critical role in higher brain activities such as cognition and mood (Janowsky, 2006; Kandel, 2001). Both subcutaneous and oral administration of the BPA significantly decreased the number of prefrontal spine synapses by 49.1 and 49.8% respectively and by 36.2 and 23.8% respectively, in the CA1 area. Spine synapse levels after subcutaneous versus oral treatments were not significantly different (Hajszan and Leranth, 2010). It seems that BPA according to remodeling of prefrontal and hippocampal spine synapses impair learning and memory in the present study.

5. CONCLUSION

In conclusion, our data showed that BPA administration at 50 mg kg^{-1} day can influence memory retention and in dose 100 and 150 mg kg^{-1} day can disrupt passive avoidance learning and memory in the shuttle box model. BPA has mixed estrogenic agonist/antagonist properties (Welshons *et al*., 2006). In the present study it seems that BPA has estrogen antagonist property so, it impair learning and memory.

6. ACKNOWLEDGMENT

This study was financially supported by Iran National Science Foundation (INSF).

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