Published in final edited form as:

J Comp Neurol. 2015 June 1; 523(8): 1248–1257. doi:10.1002/cne.23735.

Low circulating levels of bisphenol-A induce cognitive deficits and loss of asymmetric spine synapses in dorsolateral prefrontal cortex and hippocampus of adult male monkeys

John D Elsworth¹, James D Jentsch², Stephanie M Groman¹, Robert H Roth, D. Eugene Redmond Jr^{1,3}, and Csaba Leranth^{4,5}

¹Department of Psychiatry, Yale University, School of Medicine, New Haven, Connecticut, USA.

²Department of Psychology and Psychiatry & Biobehavioral Sciences, University of California, Los Angeles, USA.

³Department of Neurosurgery, Yale University, School of Medicine, New Haven, Connecticut, USA.

⁴Department of Obstetrics, Gynecology & Reproductive Sciences Yale University, School of Medicine, New Haven, Connecticut, USA.

⁵Department of Neurobiology, Yale University, School of Medicine, New Haven, Connecticut, USA.

Abstract

Bisphenol-A (BPA) is widely used in the manufacture of plastics, epoxy resins and certain paper products. A majority of the population in the developed world is routinely exposed to BPA from multiple sources and has significant circulating levels of BPA. Although BPA is categorized as an endocrine disruptor with a growing literature on adverse effects, it is uncertain whether cognitive dysfunction is induced in humans by exposure to BPA. The present study examined the impact of BPA in primate brain by exposing adult male vervet monkeys for 4 weeks continuously to circulating levels of BPA that were in the range measured in studies of humans environmentally exposed to BPA. This regimen of exposure to BPA decreased both working memory accuracy and the number of excitatory synaptic inputs on dendritic spines of pyramidal neurons in two brain regions that are necessary for working memory (prefrontal cortex and hippocampus). These observed behavioral and synaptic effects ameliorated following withdrawal from BPA. As Old world monkeys (e.g., vervets) and humans share some uniquely primate morphological, endocrine and cognitive traits, this study indicates the potential for significant cognitive disruption following exposure of humans to BPA.

Corresponding author: Dr JD Elsworth, Yale University School of Medicine, Department of Psychiatry, 300 George Street, New Haven, CT 06511. Phone: (203)-785-6768. Fax: (203)-785-7357. john.elsworth@yale.edu.

Role of authors All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: JDE, JDJ, RHR, DER, CL. Acquisition of data: JDE, JDJ, CL. Analysis and interpretation of data: JDE, SMG, CL. Drafting of manuscript: JDE. Revision of manuscript for important intellectual content: JDJ, SMG, DER, RHR, CL. Statistical analysis: JDE, SMG. Obtained funding: JDE, CL. Study supervision: JDE, JDJ, RHR, CL.

Conflict of Interest Statement The authors declare that there are no conflicts of interest regarding this research article.

Keywords

dopamine; endocrine disruptor; working memory

Introduction

Bisphenol A (BPA) is currently one of the highest production volume chemicals in the world, with 5 million metric tons produced annually (CDC, 2013) with continued growth anticipated in the upcoming years. BPA has been widely used since the 1960s in the manufacture of polycarbonate and polyvinylchloride plastics, epoxy resins and certain paper products, and it is now known that a large majority of people in the developed world are routinely exposed to BPA from multiple sources, including food and drink packaging, protective coatings on metal food cans, dental composites, thermal paper, and some water supply pipes (Chapin et al., 2008; Vandenberg et al., 2013). Alternatives to BPA for most of these applications do not perform as well, or are significantly more expensive, or have their own toxicity (Liao et al., 2012) and, thus, are not widely used. BPA is pharmacologically active and is classified as an endocrine disruptor, with its most established effects being interactions at subtypes of estrogen receptors (Rubin, 2011). It is important therefore to note that the circulating levels of BPA measured in humans are in the range (0.5-2 ng/ml) (Vandenberg et al., 2010) where pharmacological effects could occur (Vandenberg et al., 2012). In fact, there are several studies in rodents demonstrating that BPA can induce biochemical alterations in reproductive organs and brain (Welshons et al., 2006; Hajszan and Leranthe, 2010; Rubin, 2011). Presumably as a result of its CNS actions, some altered behaviors, such as learning and memory, have been observed following exposure of rodents to BPA (reviewed by Wolstenholme et al., 2011), although in all these studies BPA was administered as a bolus treatment and the circulating levels of BPA were not measured.

In view of distinct neuroendocrine differences among species (Melmed and Conn, 2005) and species differences in response to BPA (Vandenberg et al., 2012), we have been particularly interested in the effects of BPA in non-human primates, as such studies provide the most reliable indicator of the potential impact of BPA on health in humans. Indeed, Old world monkeys and humans share certain uniquely primate morphological, endocrine and cognitive traits (Lacreuse and Herndon, 2009). We have previously shown in female monkeys that a loss of asymmetric (excitatory) spine synapses in hippocampus and prefrontal cortex is induced by ovariectomy, and that the recovery of spine synapse numbers achieved by restoration of estradiol is blocked by concurrent and continuous BPA exposure (Leranthe et al., 2008a). The vast majority of asymmetric synapses in prefrontal cortex and hippocampus are located on dendritic spines of pyramidal neurons (Nimchinsky et al., 2002; Calabrese et al., 2006), and remodeling of spine synapses in prefrontal cortex and hippocampus plays a critical role in cognition, learning and memory (Kasai et al., 2003; Blanpied and Ehlers, 2004; Gonzalez-Burgos, 2012). Thus, the ability of BPA to interfere with spine synapse formation suggests that its exposure may induce profound neurocognitive effects in non-human primates. In the present study we tested this hypothesis by determining whether continuous exposure of adult male monkeys to BPA in a dose that achieves circulating levels of BPA typically measured in humans, produces loss of

asymmetric spine synapses in prefrontal cortex and hippocampus, and if so, whether these changes are associated with working memory deficits that depend on these brain regions (Goldman-Rakic, 1995; Axmacher et al., 2007; Fuster, 2008).

Materials and Methods

Animals

Sixteen young adult male St. Kitts African vervet monkeys (*Chlorocebus sabaeus*) were housed at the St Kitts Biomedical Research Foundation facilities. Monkeys were fed Harlan Teklad New Iberia Primate Diet (#8773) supplemented with seasonal local fruits. Water was constantly available, and delivered through pipes that do not contain BPA. Protocols were reviewed and approved in advance by the Animal Care and Use Committees of both Yale University and St Kitts Biomedical Research Facility; all studies were conducted in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Bisphenol-A treatment

Deuterium-labeled BPA (d-BPA, CDN Isotopes, Quebec, Canada) was used in these studies because it can be clearly distinguished from BPA by liquid chromatography-mass spectrometry (LC-MS), thus eliminating concern about potential BPA contamination from materials used in the preparation, handling or shipment of samples (Volkel et al., 2002; Taylor et al., 2011). As the goal of the study was to examine the synaptic and behavioral effects of environmentally-relevant circulating levels of BPA, we administered BPA from a subcutaneous minipump that ensured a constant and reliable delivery of BPA (Vandenberg et al., 2014). Our previous studies have confirmed that in adult male rats, administration of BPA by either subcutaneous injection or by oral gavage elicits a loss in number of spine synapses in prefrontal cortex and CA1 region of hippocampus (Hajszan and Leranth, 2010). d-BPA was delivered subcutaneously in a propylene glycol vehicle from an osmotic minipump (Alzet model 2ML4, Durect Corp., Cupertino, CA) to achieve a dose of 50 µg/kg /day for 30 days. The current oral Reference Dose for BPA is 50 µg/kg/day (U.S. Environmental Protection Agency, 1993). The minipumps were placed subcutaneously in the upper back region while animals were anesthetized. Each monkey received a minipump; half of the monkeys received d-BPA while the other half received vehicle from the minipump. The mean weight of the monkeys in each group was the same (5.5 kg). After 30 days the minipumps were removed from all animals.

Working Memory Performance

Working memory maintenance was assessed in monkeys using a two-choice spatial delayed response task (James et al., 2007). A custom-designed Wisconsin General Test apparatus fitted directly on to the cage, where an opaque screen separated the monkey from two equally spaced wells that could be covered to conceal rewards (i.e., a small piece of banana, grape or apple). A trial began when the screen was raised and the monkey watched the experimenter bait one of the two wells. The reward was concealed from view (using a small object placed directly over both wells) and the screen lowered and immediately raised (a ~0 s delay). Monkeys made a response by displacing one of the objects and, when a correct

response was made, allowed to retrieve the reward. The next trial began following a 20 s inter-trial interval. Monkeys completed 30 trials per day where delays and rewarded wells were counterbalanced and randomized across trials on each testing day

Initially all 16 untreated monkeys were trained to perform the cognitive task until each had achieved 80% success rate in retrieving the food reward from the correct well on the 0 sec delay condition. Monkeys were then exposed to sessions where the delay was increased to 2 s, then to 4 s. The “baseline” working memory capacity of monkeys was assessed during two consecutive variable delay sessions using the 0, 2, and 4 s delays. During these sessions to determine baseline performance, 1/3 of trials involved a ~0 s delay, 1/3 of trials involved a ~2 s delay and 1/3 of trials involved a ~4 s delay and the order of particular delays was randomized during the session. After analysis of working memory accuracy, monkeys were divided into 2 equally sized matching groups and osmotic minipumps containing either BPA or vehicle were implanted. One week following insertion of the osmotic BPA pumps, working memory capacity was reassessed in each group of monkeys during two variable delay sessions, applying the same delays that were used during the baseline assessments. At 4 weeks after implantation of osmotic minipumps, additional variable delay sessions were collected, where delays were increased to 0, 4, and 8 s, to theoretically increase the cognitive demands of the task. Following these behavioral assessments, half of the monkeys in each group (“BPA-chronic group” and vehicle) were euthanized and pumps were removed from the remaining monkeys. Two weeks later additional variable delay sessions using the 0, 4, and 8 s delays were collected in the remaining monkeys (“BPA-recovery group” and vehicle: n=4). The monkeys in the BPA-recovery group were euthanized 4 weeks after removal of the osmotic minipump. Our dependent measures across all variable delay sessions were the mean number of correct responses and the mean number of omissions in each group. Accuracy (percent of correct responses made over the total number of completed trials) was calculated across each of the delays for each variable delay session.

Bisphenol assay

Venous blood was collected at 1 and 4 weeks after insertion of minipumps, and unconjugated d-BPA concentration (i.e., the “free”, pharmacologically active form) was measured in plasma by LC-MS analysis as described before (Elsworth et al., 2013a). Briefly, proteins were precipitated by acetonitrile and methanol (2:1), and after centrifugation, the clear supernatant reduced to dryness under vacuum. The residue was reconstituted by mixing with 60% methanol, and an aliquot separated by reverse phase HPLC, with detection of d-BPA by selected ion monitoring of the mass-charge ratio 239. Quantification was made by reference to a concentration range of 10 external standards of d-BPA.

Postmortem sample collection

After intravenous administration of an overdose of sodium pentobarbital, brains of 3 animals from each group (BPA-chronic, BPA-recovery and vehicle) were perfused with cold saline, then removed and cut into 4 mm coronal sections as described (Elsworth et al., 2008). Specific regions were punch dissected from the fresh slices on a refrigerated surface, placed in cryotubes and frozen in liquid nitrogen. The remainder of each section was post-fixed at 4 C, either in 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4 for 24 hours, or in a

series of fixatives containing increasing concentrations of paraformaldehyde and glutaraldehyde in 0.1 M phosphate buffer pH 7.4 over 4 days. This latter fixation technique enabled spine synapse counts to be made by electron microscopy in tissue from the immersion fixation process, as described previously (Elsworth et al., 2013b). Fixed samples were stored in 0.1M phosphate buffer pH 7.4 containing 0.1% sodium azide at 4 C. The brain of 1 animal from each of the 3 groups was perfused with room temperature saline, followed by 4% paraformaldehyde and 1% glutaraldehyde in 0.1M phosphate buffer pH 7.4, followed by overnight fixation in 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4 at 4 C, before storage in 0.1M phosphate buffer pH 7.4 containing 0.1% sodium azide at 4 C. These 3 brains were perfusion-fixed instead of immersion-fixed to allow a more detailed examination of the ultrastructure by electron microscopy.

Spine Synapses

The numbers of asymmetric (excitatory) spine synapses in CA1 stratum radiatum and layers 2/3 of Walker's area 46 of DLPFC were calculated using an unbiased electron microscopic stereological approach as published previously (Leranth et al., 2008b; Miettinen et al., 2012). Layers 2/3 of DLPFC were chosen for analysis because: a) they are well positioned to exert powerful effects on cognition, sending projections to associational cortical regions in addition to innervating layer 5 neurons (Pucak et al., 1996; Song et al., 2012), b) asymmetric spine synapse number in these layers is significantly correlated with working memory performance in primates (Peters et al., 2008), c) in primates there is a relatively dense DA innervation of layers 2/3 in DLPFC (Kroner et al., 2007), which appears to regulate asymmetric spine synapse number (Elsworth et al., 2013b), and d) asymmetric spine number in layers 2/3 of DLPFC in adult female monkeys is known to be susceptible to BPA (Leranth et al., 2008a). The CA1 subfield of hippocampus was studied because: a) it is known that this region is critical for memory formation (Bird and Burgess, 2008) and b) asymmetric spine synapse number in this region in adult monkeys is particularly sensitive to BPA and to changes in gonadal hormone levels (Leranth et al., 2008a; Hajszan and Leranth, 2010). Synapses on spines in DLPFC and CA1 were identified by the presence of synaptic densities, as well as by the absence of mitochondria, microtubules, and synaptic vesicles. Boutons were recognized by the presence of synaptic vesicles and mitochondria. Asymmetric spine synapses were identified between spines and boutons by the characteristically electron dense postsynaptic density (see Figure 1).

DA assay

In view of our finding indicating that DA can regulate the number of asymmetric synapses (Elsworth et al., 2013b), DA concentration was measured in tissue dissected from the DLPFC, dorsal caudate nucleus and dorsal putamen. The method used for determination of DA concentration is described in detail elsewhere (Morrow et al., 2011). Briefly, frozen tissues were first sonicated in cold perchloric acid containing dihydroxybenzylamine as internal standard. After centrifugation, catechols in the supernatant were adsorbed on an alumina column at pH 8.2, then eluted in dilute acid and finally separated by reverse-phase isocratic HPLC. DA was detected electrochemically, and quantified with respect to internal and external standards. The centrifuged tissue pellet was digested with sodium hydroxide,

and its protein concentration measured using the Lowry method. DA concentrations in tissue samples were calculated as ng per mg protein.

Research Resource Identifiers (RRIDs)

Tools, Software, and Databases: Statistical analyses were conducted with either “IBM SPSS Statistics for Macintosh, Version 21.0 (Chicago, IL)” or “GraphPad Prism version 6.0d for Macintosh, GraphPad Software, Inc. (La Jolla, CA).”

Statistics

Group comparisons were made using non-parametric statistics, and details of individual results are provided in the appropriate sections of Results. Significance was achieved when $p < 0.05$, and lower probabilities were regarded as not significant (NS).

Results

BPA levels

At 1 week after subcutaneous implantation of the pump, the mean plasma level of d-BPA was 0.8 ± 0.1 ng/ml, and just before euthanasia the mean value had fallen to 0.3 ± 0.2 ng/ml. In plasma samples from monkeys that did not receive an implant of d-BPA, there was no peak in the LC-MS chromatogram that corresponded to the retention time and mass-to-charge ratio of d-BPA. Thus, the minipump BPA administration protocol achieved circulating levels of BPA in monkeys that were the range found in surveys of routine human exposure to BPA (Vandenberg et al., 2010).

Working Memory Performance

Accuracy in the two-choice spatial delay response task was examined using Friedman's test with the accuracy before (baseline) and after osmotic pump implantation (1 week and 4 weeks) at each of the delays (0s, middle delay, long delay) entered as repeated measures and treatment (BPA or vehicle) as the between-subjects factor. Significant effects were followed up using Wilcoxon Signed Rank tests. In BPA-exposed monkeys, there was a significant effect of time on accuracy in the 0 s delay ($\chi^2=7.46$; $p=0.02$) and middle delay ($\chi^2=6.65$; $p=0.03$; Figure 2A), but not at the longest delay ($\chi^2=4.32$; $p=0.12$). We then examined whether these changes in accuracy occurred at the assessments collected one or four weeks following BPA osmotic pump implantation. Compared to baseline accuracy, accuracy at the 0 s and middle delay was significantly lower one week following BPA osmotic pump implantation (0 s delay: $Z=2.21$; $p=0.03$; middle delay: $Z=2.37$; $p=0.02$), but not four weeks following BPA osmotic pump implantation (0 s delay: $Z=1.05$; $p=0.29$; middle delay: $Z=1.40$; $p=0.16$). In vehicle-exposed monkeys, there was a trend for a decrease in accuracy at the 0 s delay ($\chi^2=6.0$; $p=0.05$), but no significant effects on accuracy at the middle ($\chi^2=0.27$; $p=0.88$) or the longest delay ($\chi^2=4.32$; $p=0.12$). The statistical analysis (above) indicated that working memory accuracy had returned to baseline levels when osmotic minipumps were removed and at the time some of the monkeys were euthanized. However, we continued to assess the performance of the remaining monkeys in case any changes occurred during the recovery period (Figure 2B). The Wilcoxon Signed Rank test showed that 2 weeks after pump removal, there was no significant change in working memory

accuracy in either group, although BPA-exposed monkeys displayed a non-significant trend-level improvement in accuracy at the middle delay ($Z=1.83$; $p=0.06$). Overall, these behavioral data indicate that BPA exposure was associated with a deficit in working memory accuracy when the plasma levels of BPA was 0.8 ng/ml (first week of exposure), but that the deficit ameliorated when the plasma level had fallen to 0.3 ng/ml (fourth week of exposure).

Spine Synapses

BPA exposure for 4 weeks (BPA-chronic group) resulted in a significant loss of spine synapses in both DLPFC and the CA1 regions of hippocampus compared with age- and sex-matched control monkeys (Figures 3-5). In addition, this BPA-induced loss of spine synapses did not completely recover during the 4 weeks after minipump removal, as the BPA-recovery group had lower numbers of spine synapses in both DLPFC and hippocampus compared with controls (Fig 5). Specifically, Kruskal-Wallis test for DLPFC reported a significant difference between the 3 groups [$p<0.0002$] with each group being significantly different from the other groups (Mann-Whitney test, $P<0.05$). Similarly, the Kruskal-Wallis test for hippocampus revealed a significant difference between the 3 groups [$p<0.0002$] with each group being significantly different from the other groups (Mann-Whitney test, $P<0.05$). The main findings from measurements of asymmetric spine synapses in this study were that BPA exposure was associated with decreased number of spine synapses in DLPFC and hippocampus, and that full recovery did not occur in the 4 weeks following cessation of BPA administration.

DA levels

DA levels in the DLPFC were 38% lower in the BPA-exposed monkeys compared with controls, but this difference was not statistically significant. It should be noted though that in this analysis there were only 3 animals in each group and that DA levels in DLPFC are inherently quite variable between animals, so there was little statistical power to detect anything but a very sizeable loss of DA. In fact, if historical controls for DA levels in DLPFC are added to the analysis (Elsworth et al., 2013b), then the values from the BPA-exposed monkeys were significantly lower than these controls. Analysis of DA levels in caudate nucleus and putamen by two-way ANOVA revealed significant effects of BPA and region with no significant interaction between these factors. However, non-parametric statistics are more appropriate for this analysis, and this approach did not produce any significant results. Together these data suggest a possible interaction of BPA with DA neurons innervating DLPFC and striatum that deserves further study.

Discussion

The present study involved constant exposure of monkeys to BPA for 4 weeks such that the circulating level of unconjugated BPA was within the range reported to be present in most people in developed countries. This administration protocol resulted in a loss of asymmetric spine synapses in the DLPFC and CA1 region of hippocampus and a deficit in working memory performance compared with control monkeys. These data demonstrate that BPA exposure has the potential to induce significant cognitive disruption in humans.

There is significant controversy about the actual extent of exposure to BPA and the health risks of such exposures (Vandenberg et al., 2013). Toxicokinetic models have predicted that the amount of BPA typically consumed by humans should not produce detectable circulating levels of free BPA, based on the assumption that orally delivered BPA is rapidly conjugated in the liver and thus inactivated. However this assumption belies the reports from many studies of bioactive BPA levels in the blood of most subjects (Vandenberg et al., 2010). There are several possible resolutions to this quandary. It is conceivable that the assays of serum or plasma BPA levels are not reliable, although great care has been taken by investigators to avoid false positives, and studies in which subjects purposefully alter their intake of BPA-containing foods have shown concomitant alteration in measured blood levels of BPA. Alternatively, people may ingest more BPA than originally estimated or be exposed to BPA by non-oral routes that would not be subject to first-pass hepatic metabolism, for example by transdermal absorption from BPA-containing items such as thermal paper or inhalation from dust particles containing BPA. Another possibility is that to some extent, conjugated BPA is de-conjugated in vivo and this contributes to free BPA that is detected in blood. It is clear though from the present study that constant exposure of non-human primates to BPA at blood levels measured in humans leads to changes in synaptic plasticity and measureable cognitive deficits. Thus there appears to be important implications of the current study for most individuals in developed regions of the world.

The extent of loss of spine synapses in both DLPFC and hippocampus was substantial. A spatial working memory behavioral task was used in the present study and it is known that the networks involving primate DLPFC are especially critical for spatial working memory processes, by temporarily storing the spatial information required to complete the task (Goldman-Rakic, 1995). Although hippocampus is prominently associated with long-term declarative memory, there is also strong support for the recruitment of hippocampus during working memory tasks (Axmacher et al., 2007). Thus, the BPA-induced losses of spine synapses in DLPFC and CA1 region of hippocampus probably both contributed to the observed working memory deficits.

The cognitive deficit induced by BPA exposure in the present study dissipated during the 2 weeks following removal of the minipump that delivered BPA to the monkeys (BPA-recovery group). This finding demonstrates that the cognitive deficit associated with BPA exposure is reversible, although the exact timing of the recovery is not yet known. It is also unclear whether intermittent BPA exposure would provoke similar behavioral changes to those that we have observed with constant administration. An unexpected finding in this study was that the number of spine synapses in both DLPFC and hippocampus in the BPA-recovery group was significantly lower than control values. This result indicates that the impact of BPA on spine synapses in these 2 brain regions lasts at least 4 weeks following cessation of BPA exposure, even though performance in the cognitive test normalizes within 2 weeks removal of the minipump that delivered BPA. It is not known why the number of spine synapses did not recover fully following cessation of BPA administration or if the numbers would eventually return to control levels. One possibility is that compensatory changes in the neural networks subserving working memory resulted in apparently normal performance on the task while the number of spine synapses in DLPFC and/or hippocampus was significantly lower than in controls. Alternatively, it is possible that apparently normal

working memory in the 2-well spatial delayed task can be achieved with approximately a 20% loss of spine synapses in DLPFC and hippocampus. In addition it should be noted that even though there was no deficit in performance of the 2-well spatial delayed task at 4 weeks after BPA administration, there may have been deficits in other behaviors or endpoints that were not monitored in this study.

An important variable in the overall impact of BPA exposure may be the age of the population being examined. Indeed, there is much concern over the effects of BPA on the developing brain, as this is when a strict sequence of spatial and temporal biochemical signals shapes the functional organization of brain and so it is a time when there is great potential for a toxin to cause long-lasting damage. In fact, we have found that administration of BPA to pregnant monkeys induces loss of asymmetric spine synapses in hippocampus and reduction in the number of tyrosine hydroxylase-immunostained midbrain DA neurons in the offspring (Elsworth et al., 2013a). In contrast, we found that exposure to relatively high levels of BPA did not affect spine synapses or midbrain DA neurons in juvenile monkeys (Elsworth et al., 2013a). Thus, our recent studies have demonstrated an impact of BPA on synaptic plasticity at the prenatal and adult stages of life, but not during the juvenile period (present study and Leranth et al., 2008a; Elsworth et al., 2013a). These different outcomes in monkeys illustrate the potential importance of age as a variable in the vulnerability of the brain to the effects of BPA. As we discussed before, this age-dependent resistance to BPA in juvenile monkeys may be due to differences in hormone levels and their interactions at that stage of life. Alternatively it may be connected to the existence of supernumerary dendritic spines in the cerebral cortex during the pre-adult period in primates. During puberty in primates, selective elimination of the initially overproduced number of synaptic spine in cerebral cortex occurs, and this synaptic pruning continues into early adulthood (Bourgeois et al., 1994; Petanjek et al., 2011). Thus, synaptic density in prefrontal cortex regions peaks at about the juvenile stage of development, and the resistance of spine synapse number to BPA at this age may conceivably be related to an increased resilience of spine synapse number to BPA in the pre-pruning period. This contention is supported by the evidence that some of the actions of BPA may be DA-dependent (Masuo and Ishido, 2011; Elsworth et al., 2013a) together with the observation that there is a different sensitivity and distribution of DA receptors in adolescence compared with adulthood (Petanjek et al., 2011; Selemon, 2013). The impact of BPA on the fetal and adult CNS has led to speculations for a role of BPA exposure in neurodegenerative disorders, in addition to memory and cognitive disorders (Masuo and Ishido, 2011). Interestingly, a recent review of the evidence for an effect of BPA on humans found 75 studies linking exposure to adverse perinatal, childhood, and adult health effects (Rochester, 2013).

Loss of asymmetric spine synapses in DLPFC in monkeys has been firmly linked with a reduced frequency of spontaneous excitatory postsynaptic currents and impairment in prefrontal cortex-dependent cognitive tasks (Luebke et al., 2004; Peters et al., 2008). In fact, the number of asymmetric synapses correlates with working memory performance in monkeys (Peters et al., 2008; Elsworth et al., 2015). Thus, the loss of asymmetric synapses in DLPFC in the present study is a likely significant contributor to the cognitive deficits found in the BPA treated monkeys. The upstream biochemical events that drive the

reduction in spine synapse number are not known. One candidate for regulating an alteration of asymmetric spine synapse formation in DLPFC is dysregulation of DA transmission. Support for this contention derives from our studies in MPTP-treated monkeys where there is a preferential loss of DA concentration in targets innervated by midbrain DA neurons, which includes DLPFC, and a loss of asymmetric spine synapses (Elsworth et al., 2013b) together with cognitive deficits (Taylor et al., 1990). Thus, it is possible that dopaminergic alterations played a role in the observed loss of spine synapses and cognitive deficits. However, BPA has a complicated pharmacology and many targets for its action have been identified in different assay systems, including epigenetic changes and pathways and genes regulated by estradiol and thyroid hormones (Hajszan and Leranth, 2010; Rubin, 2011; Alonso-Magdalena et al., 2012). Future studies will be necessary to delineate accurately the mechanism underlying the effect of BPA on cognition.

In conclusion, this study reveals that significant behavioral and synaptic effects result from exposure of adult male non-human primates to blood levels of BPA that are in the range found in most people in the developed world. Specifically, constant administration of relatively low levels of BPA induced a marked loss of excitatory synaptic inputs on dendrites of pyramidal neurons in DLPFC and hippocampus, and a deficit in working memory accompanied these changes in synaptic plasticity. While these findings provide a clear warning of potential harmful effects of BPA in young adults, it is quite feasible that the impact of BPA may be magnified in the developing or aged brain.

Other Acknowledgements

We thank Klara Szigeti-Buck and Feng-Pei Chen for their excellent technical expertise at Yale University, and the staff at the St Kitts Biomedical Research Foundation for their invaluable assistance in the treatment and care of animals.

Support: NIEHS ES017013 to CL, NS056181 to JDE.

Literature Cited

- Alonso-Magdalena P, Ropero AB, Soriano S, Garcia-Arevalo M, Ripoll C, Fuentes E, Quesada I, Nadal A. Bisphenol-A acts as a potent estrogen via non-classical estrogen triggered pathways. *Molecular and cellular endocrinology*. 2012; 355(2):201–207. [PubMed: 22227557]
- Axmacher N, Mormann F, Fernandez G, Cohen MX, Elger CE, Fell J. Sustained neural activity patterns during working memory in the human medial temporal lobe. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2007; 27(29):7807–7816. [PubMed: 17634374]
- Bird CM, Burgess N. The hippocampus and memory: insights from spatial processing. *Nature reviews Neuroscience*. 2008; 9(3):182–194.
- Blanpied TA, Ehlers MD. Microanatomy of dendritic spines: emerging principles of synaptic pathology in psychiatric and neurological disease. *Biol Psychiatry*. 2004; 55(12):1121–1127. [PubMed: 15184030]
- Bourgeois JP, Goldman-Rakic PS, Rakic P. Synaptogenesis in the prefrontal cortex of rhesus monkeys. *Cereb Cortex*. 1994; 4(1):78–96. [PubMed: 8180493]
- Calabrese B, Wilson MS, Halpain S. Development and regulation of dendritic spine synapses. *Physiology (Bethesda)*. 2006; 21:38–47. [PubMed: 16443821]
- CDC. Biomonitoring Survey: Bisphenol A. 2013. Available from: http://www.cdc.gov/biomonitoring/BisphenolA_BiomonitoringSummary.html
- Chapin RE, Adams J, Boekelheide K, Gray LE Jr, Hayward SW, Lees PS, McIntyre BS, Portier KM, Schnorr TM, Selevan SG, Vandenberg JG, Woskie SR. NTP-CERHR expert panel report on the

reproductive and developmental toxicity of bisphenol A. Birth defects research Part B, Developmental and reproductive toxicology. 2008; 83(3):157–395.

- Elsworth JD, Groman SM, Jentsch JD, Leranath C, Redmond DE Jr. Roth RH. Primate phencyclidine model of schizophrenia: sex-specific effects on cognition, BDNF, spine synapses and dopamine turnover in prefrontal cortex. *Int J Neuropsychopharmacol*. 2015 in press.
- Elsworth JD, Jentsch JD, Morrow BA, Redmond DE Jr. Roth RH. Clozapine normalizes prefrontal cortex dopamine transmission in monkeys subchronically exposed to phencyclidine. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 2008; 33(3):491–496. [PubMed: 17507917]
- Elsworth JD, Jentsch JD, Vandevort CA, Roth RH, Jr DE, Leranath C. Prenatal exposure to bisphenol A impacts midbrain dopamine neurons and hippocampal spine synapses in non-human primates. *Neurotoxicology*. 2013a; 35:113–120. [PubMed: 23337607]
- Elsworth JD, Leranath C, Redmond DE Jr. Roth RH. Loss of asymmetric spine synapses in prefrontal cortex of motor-asymptomatic, dopamine-depleted, cognitively impaired MPTP-treated monkeys. *Int J Neuropsychopharmacol*. 2013b; 16(4):905–912. [PubMed: 22947206]
- Fuster, J. *The Prefrontal Cortex*. Elsevier; 2008.
- Goldman-Rakic PS. Cellular basis of working memory. *Neuron*. 1995; 14(3):477–485. [PubMed: 7695894]
- Gonzalez-Burgos I. From synaptic transmission to cognition: an intermediary role for dendritic spines. *Brain and cognition*. 2012; 80(1):177–183. [PubMed: 22516877]
- Hajszan T, Leranath C. Bisphenol A interferes with synaptic remodeling. *Frontiers in neuroendocrinology*. 2010; 31(4):519–530. [PubMed: 20609373]
- James AS, Groman SM, Seu E, Jorgensen M, Fairbanks LA, Jentsch JD. Dimensions of impulsivity are associated with poor spatial working memory performance in monkeys. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2007; 27(52):14358–14364. [PubMed: 18160643]
- Kasai H, Matsuzaki M, Noguchi J, Yasumatsu N, Nakahara H. Structure-stability-function relationships of dendritic spines. *Trends in neurosciences*. 2003; 26(7):360–368. [PubMed: 12850432]
- Kroner S, Krimer LS, Lewis DA, Barrionuevo G. Dopamine increases inhibition in the monkey dorsolateral prefrontal cortex through cell type-specific modulation of interneurons. *Cereb Cortex*. 2007; 17(5):1020–1032. [PubMed: 16772311]
- Lacreuse, A.; Herndon, J. Nonhuman primate models of cognitive aging. In: Bizon, J.; Woods, A., editors. *Animal Models of Cognitive Aging*. Humana Press; New York: 2009. p. 29-58.
- Leranath C, Hajszan T, Szigeti-Buck K, Bober J, MacLusky NJ. Bisphenol A prevents the synaptogenic response to estradiol in hippocampus and prefrontal cortex of ovariectomized nonhuman primates. *Proceedings of the National Academy of Sciences of the United States of America*. 2008a; 105(37):14187–14191. [PubMed: 18768812]
- Leranath C, Szigeti-Buck K, MacLusky NJ, Hajszan T. Bisphenol A prevents the synaptogenic response to testosterone in the brain of adult male rats. *Endocrinology*. 2008b; 149(3):988–994. [PubMed: 18048497]
- Liao C, Liu F, Alomirah H, Loi VD, Mohd MA, Moon HB, Nakata H, Kannan K. Bisphenol S in urine from the United States and seven Asian countries: occurrence and human exposures. *Environmental science & technology*. 2012; 46(12):6860–6866. [PubMed: 22620267]
- Luebke JI, Chang YM, Moore TL, Rosene DL. Normal aging results in decreased synaptic excitation and increased synaptic inhibition of layer 2/3 pyramidal cells in the monkey prefrontal cortex. *Neuroscience*. 2004; 125(1):277–288. [PubMed: 15051166]
- Masuo Y, Ishido M. Neurotoxicity of endocrine disruptors: possible involvement in brain development and neurodegeneration. *Journal of toxicology and environmental health Part B, Critical reviews*. 2011; 14(597):346–369.
- Melmed, S.; Conn, P. *Endocrinology. Basic and Clinical Principles*. Humana Press; Totowa, NJ: 2005.
- Miettinen R, Hajszan T, Riedel A, Szigeti-Buck K, Leranath C. Estimation of the total number of hippocampal CA1 pyramidal neurons: New methodology applied to helpless rats. *Journal of neuroscience methods*. 2012; 205(1):130–138. [PubMed: 22230770]

- Morrow BA, Roth RH, Redmond DE, Elsworth JD. Impact of methamphetamine on dopamine neurons in primates is dependent on age: implications for development of Parkinson's disease. *Neuroscience*. 2011; 189:277–285. [PubMed: 21640165]
- Nimchinsky EA, Sabatini BL, Svoboda K. Structure and function of dendritic spines. *Annu Rev Physiol*. 2002; 64:313–353. [PubMed: 11826272]
- Petanjek Z, Judas M, Simic G, Rasin MR, Uylings HB, Rakic P, Kostovic I. Extraordinary neoteny of synaptic spines in the human prefrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108(32):13281–13286. [PubMed: 21788513]
- Peters A, Sethares C, Luebke JI. Synapses are lost during aging in the primate prefrontal cortex. *Neuroscience*. 2008; 152(4):970–981. [PubMed: 18329176]
- Pucak ML, Levitt JB, Lund JS, Lewis DA. Patterns of intrinsic and associational circuitry in monkey prefrontal cortex. *The Journal of comparative neurology*. 1996; 376(4):614–630. [PubMed: 8978474]
- Rochester JR. Bisphenol A and human health: a review of the literature. *Reprod Toxicol*. 2013; 42:132–155. [PubMed: 23994667]
- Rubin BS. Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. *The Journal of steroid biochemistry and molecular biology*. 2011; 127(192):27–34. [PubMed: 21605673]
- Selemon LD. A role for synaptic plasticity in the adolescent development of executive function. *Translational psychiatry*. 2013; 3:e238. [PubMed: 23462989]
- Song, D.; Opris, I.; Chan, RH.; Marmarelis, VZ.; Hampson, RE.; Deadwyler, SA.; Berger, TW. Functional connectivity between Layer 2/3 and Layer 5 neurons in prefrontal cortex of nonhuman primates during a delayed match-to-sample task. *Conference proceedings : Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE Engineering in Medicine and Biology Society Conference*; 2012; 2012. p. 2555-2558.
- Taylor JA, Vom Saal FS, Welshons WV, Drury B, Rottinghaus G, Hunt PA, Toutain PL, Laffont CM, VandeVoort CA. Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: relevance for human exposure. *Environmental health perspectives*. 2011; 119(4):422–430. [PubMed: 20855240]
- Taylor JR, Roth RH, Sladek JR Jr, Redmond DE Jr. Cognitive and motor deficits in the performance of an object retrieval task with a barrier-detour in monkeys (*Cercopithecus aethiops sabaeus*) treated with MPTP: long-term performance and effect of transparency of the barrier. *Behavioral neuroscience*. 1990; 104(4):564–576. [PubMed: 2206426]
- U.S. Environmental Protection Agency. Bisphenol A. 1993. Available from: <http://www.epa.gov/iris/subst/0356.htm>
- Vandenberg LN, Chahoud I, Heindel JJ, Padmanabhan V, Paumgartten FJ, Schoenfelder G. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. *Environmental health perspectives*. 2010; 118(8):1055–1070. [PubMed: 20338858]
- Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr, Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV, Zoeller RT, Myers JP. Hormones and endocrine disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocrine reviews*. 2012; 33(3):378–455. [PubMed: 22419778]
- Vandenberg LN, Hunt PA, Myers JP, Vom Saal FS. Human exposures to bisphenol A: mismatches between data and assumptions. *Reviews on environmental health*. 2013; 28(1):37–58. [PubMed: 23612528]
- Vandenberg LN, Welshons WV, Vom Saal FS, Toutain PL, Myers JP. Should oral gavage be abandoned in toxicity testing of endocrine disruptors? *Environmental health : a global access science source*. 2014; 13(1):46. [PubMed: 24961440]
- Volkel W, Colnot T, Csanady GA, Filser JG, Dekant W. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. *Chemical research in toxicology*. 2002; 15(10):1281–1287. [PubMed: 12387626]
- Welshons WV, Nagel SC, vom Saal FS. Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. *Endocrinology*. 2006; 147(6 Suppl):S56–69. [PubMed: 16690810]

Wolstenholme JT, Rissman EF, Connelly JJ. The role of Bisphenol A in shaping the brain, epigenome and behavior. *Hormones and behavior*. 2011; 59(3):296–305. [PubMed: 21029734]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1. Identification of asymmetric spine synapses. Electron micrograph shows an asymmetric synaptic contact on a pyramidal cell apical dendritic (Pd) spine (Sp) in CA1 region of hippocampus. Note the spine apparatus (arrow). Asymmetric synapses on dendritic shafts (D) were not counted. Bar scale: 500 nm.

Figure 2.

2A - Accuracy of monkeys performing a variable spatial delay response task before (Baseline), and at 1-week and 4-weeks after implantation of an osmotic pump containing either vehicle or BPA. Data show median values with interquartile ranges in parentheses. * indicates performance was significantly different from baseline at $p < 0.05$. **Fig 2B** - Accuracy of monkeys in a variable spatial delay response task 4-weeks following osmotic pump implantation containing either vehicle or BPA and 2 weeks after osmotic pump removal.

Figure 3.

Electron micrograph of the DLPFC from a control monkey (A) and BPA-exposed monkey (B), illustrating loss of spine synapses in the treated animal. Abbreviations: D, dendrite; arrows point to spine synapses.

Figure 4.

Electron micrograph of the CA1 region of hippocampus from a control monkey (A) and BPA-exposed monkey (B), illustrating loss of spine synapses in the treated animal.

Abbreviations: D, dendrite; arrows point to spine synapses.

Figure 5.

Lower number of spine synapses in layer 2/3 of DLPFC (A) and CA1 region of hippocampus (B) of BPA-exposed monkeys compared with controls. The loss of spine synapses partially recovered during the month following BPA withdrawal. The median number of spine synapses in each group was significantly different from the value of other groups, as denoted by a different letter on each bar ($p < 0.0002$). Error bars show the interquartile range.