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Sex Differences in Microglial Colonization and Vulnerabilities to Endocrine Disruption in the Social Brain

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Abstract

During development, microglia, the resident immune cells of the brain, play an important role in synaptic organization. Microglial colonization of the developing brain is sexually dimorphic in some regions, including nuclei critical for the coordination of social behavior, suggesting steroid hormones have an influencing role, particularly estrogen. By extension, microglial colonization may be vulnerable to endocrine disruption. Concerns have been raised regarding the potential for endocrine disrupting compounds (EDCs) to alter brain development and behavior. Developmental exposure to Bisphenol A (BPA), a ubiquitous EDC, has been associated with altered sociosexual and mood-related behaviors in various animal models and children. Through a comparison of the promiscuous Wistar rat (*Rattus norvegicus*) and the socially monogamous prairie vole (*Microtus ochrogaster*), we are the first to observe that developmental exposure to the synthetic estrogen ethinyl estradiol (EE) or BPA alters the sex-specific colonization of the hippocampus and amygdala by microglia.

Keywords

vole; rat; hypothalamus; EDC; sexual differentiation; puberty

1. Introduction

Environmental factors have been heavily implicated in the etiology neurodevelopmental disorders, including psychosocial disorders, but direct evidence linking chemical exposures to social deficits or effects in brain regions critical for sociality is sparse, and no single chemical has been definitively implicated (Grandjean and Landrigan, 2014; Kalkbrenner et

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al., 2014; Landrigan et al., 2012). For example, genetic factors contribute only an estimated 30–40% of autism spectrum disorder (ASD) heritability (Landrigan et al., 2012; Sandin et al., 2014), emphasizing that it and other disorders of the social brain likely result from complex gene by environment interactions. The prevalence of ASD and other psychiatric disorders is sex-biased, suggesting that the environmental susceptibility is also likely sexually dimorphic. Rapidly emerging work has demonstrated that microglia, hormone-sensitive, resident immune cells of the brain, coordinate key aspects of early brain development and patterning thought to be altered in ASD patients, including synapse pruning. Thus, disruption of the distribution and function of microglia may be a risk factor for impaired sociality. Work in rats has shown that the colonization of the developing brain by microglia has region-specific sexually dimorphic aspects and contribute to sexually dimorphic behaviors and responses to environmental challenges (Bilbo, 2013; Schwarz et al., 2012). We hypothesize that disrupted microglial colonization of brain areas fundamental to sociality might be a mechanism by which endocrine disrupting compounds (EDCs) and other chemicals may adversely impact brain organization and, consequently, social behaviors.

To test this hypothesis we used two different species (1) rats, because the microglial colonization of the developing rat brain has already been described to some degree (Schwarz et al., 2012) and (2) prairie voles (*Microtus ochrogaster*) because they are a well-established, pro-social, animal model used for decades to elucidate the neuroendocrinology of the social brain, the evolution of social traits, and the biological basis of social disorders (McGraw and Young, 2010; Williams and Carter; Winslow et al., 1993; Young et al., 1998). Prairie voles are uniquely suited to test our hypothesis because they display more human-typical affiliative traits including pair bonding and paternal care. Importantly, for these studies, we used brain tissues from animals (rats and voles) for which we have already shown that postnatal exposure to the EDC Bisphenol A (BPA) can alter social and emotional behaviors including open field exploration (Patisaul et al., 2012; Sullivan et al., 2014). Examining brains from animals for which we also have behavioral data makes it possible for us to begin to probe the potential mechanisms by which these behavioral outcomes occur.

Microglia are highly mobile cells and in response to insult, injury, or infection, produce pro- and anti-inflammatory cytokines and chemokines (Harry and Kraft, 2008; Waisman et al., 2015). Microglial morphology changes with age and functional state (Figure 1A; reviewed in (Karperien et al., 2013)). While the neuroinflammatory role of microglia in the mature brain is reasonably well defined, their role in development is more ambiguous, but multimodal and fundamental to synaptic organization (Lenz and McCarthy, 2014; Saijo and Glass, 2011). The sexually dimorphic aspects of microglial ontogeny and actions in the developing brain suggest a coordinating role for steroid hormones (Hanamsagar and Bilbo, 2015; Schwarz et al., 2012) but this has not been definitively demonstrated. In adult microglia, estrogen is important for both basal and proinflammatory responses, while testosterone inhibits glial activation (Barreto et al., 2007; Habib and Beyer, 2015; Sierra et al., 2008). The influence of steroid hormones on developing microglia, however, remains to be elucidated. To begin to address this data gap, here we explored how developmental exposure to ethinyl estradiol (EE) alters microglial numbers in the postnatal rat dentate gyrus (DG), a region previously shown to have age-specific sexual dimorphisms in

colonization (Schwarz et al., 2012), with the hypothesis that exogenous estrogen would alter their developmental trajectory.

We also tested the hypothesis that environmental endocrine disruption can alter microglia colonization in brain subregions fundamental to sociality. BPA was selected as our EDC of choice because it is widely studied, its biological effects have been well characterized, and it has become ubiquitous in the environment and our bodies, prompting concerns about its potential health impacts on wildlife and humans, especially on neural development and behavior (FAO/WHO, 2011; NTP, 2008). It was also selected because we could use tissues from animals for which we have previously published behavioral, neural and/or BPA exposure-related data, thereby facilitating the identification of potential linkages between behavioral and central nervous system outcomes (Patisaul et al., 2012; Sullivan et al., 2014). Microglial colonization was examined in the neonatal rat DG and the adult prairie vole DG, amygdala and somatosensory cortex, the latter of which was included to test the hypothesis that BPA-related effects would be region specific. No effects of BPA on microglial colonization were expected in the somatosensory cortex because it is not considered fundamental to social behavior. To our knowledge, nothing is known about microglial colonization of the prairie vole brain and thus the results presented here provide fundamental information about the microglial colonization in this uniquely prosocial species. Here we show, for the first time, that developmental exposure to BPA or the synthetic estrogen EE have sex-specific effects on microglial colonization.

2. Materials and Methods

2.1. Animal Care and Use

Complete study design details regarding husbandry, care and animal handling are described in detail elsewhere for the rats (Patisaul et al., 2012) and the prairie voles (Sullivan et al., 2014). For both species, all work was done according to the applicable portions of the Animal Welfare Act and the U.S. Department of Health and Human Services Guide for the Care and use of Laboratory Animals. All aspects of the rat studies were approved by the Institutional Animal Care and Use Committee of North Carolina State University and all aspects of the prairie vole studies were approved by the Northeast Ohio Medical University and conducted in an Association for Assessment and Accreditation of Laboratory Animal Care approved facility affiliated with the Cushing lab at the University of Akron. As in our prior studies (Patisaul et al., 2012; Patisaul et al., 2009), and in accordance with recommended practices for EDC research (Hunt et al., 2009; Li et al., 2008; Richter et al., 2007), all animals were housed in conditions specifically designed to minimize unintended EDC exposure.

2.2. Experiment 1: Postnatal Rat Microglia

This experiment tested the hypothesis that perinatal BPA or EE would have sex-specific effects on the microglial colonization of the post-natal rat dentate gyrus (day of analysis was postnatal day (PND) 12). This brain region was chosen because prior work in rats has described the sex-specific microglial colonization of this region (Schwarz et al., 2012), but not the impact of developmental hormone manipulation. Brain tissues for these experiments

were obtained from a subset of animals in a larger study, detailed elsewhere (Patisaul et al., 2012). Briefly, on gestational day 6, four cohorts of Wistar dams (maintained on Teklad 2020 diet, Harlan) were randomly assigned to one of three groups: control, BPA or EE. BPA ((2,2-bis(4-hydroxyphenyl)propane; CAS No. 80-05-7; Lot 11909; USEPA/NIEHS standard provided to HBP); 1mg/L of water) and EE (50µg/L) were administered via drinking water. Exposure continued up through the day of sacrifice (PND 12). This dose of BPA was chosen based on prior studies utilizing this method of exposure (Fujimoto et al., 2006; Kabuto et al., 2004; Miyawaki et al., 2007) to produce serum levels in the human range. In a prior study we confirmed that, in the exposed pups, unconjugated BPA levels were less than 2 ng/ml; a range that approximates the current estimated mean serum levels in humans (Patisaul et al., 2012; Vandenberg et al., 2007).

2.2.1. Tissue Collection and Immunolabeling—Pups were sacrificed on PND 12 via rapid decapitation and the brains post-fixed overnight in 4% paraformaldehyde at 4°C with mild shaking, then an additional 24 hours in fresh 4% paraformaldehyde. All brains (no more than two per sex per litter) were then cryoprotected by incubating them for 8 hours in 4% paraformaldehyde with 30% sucrose, and then 30% buffered sucrose for 48 hours (Hoffman and Le, 2004) before rapidly freezing them on dry ice (stored at −80°C). All brains were sliced into 35 µm coronal sections and stored free floating in antifreeze (20% glycerol, 30% ethylene glycol in potassium phosphate buffer solution) at −20°C. With the guidance of the Paxinos and Watson rat brain atlas (Paxinos and Watson, 2007), for each animal, six consecutive sections per animal containing the dentate gyrus (DG; Bregma −2.64 through −3.60) were mounted and the microglia immunolabeled via standard immunohistochemistry procedures in our laboratory (Patisaul et al., 2009) using the ionized calcium-binding adaptor molecule (Iba)-1 (rabbit anti-Iba1, Wako Chemicals, Richmond, Virginia, 1:10,000) as the primary antibody and DAB as the chromagen (McCaffrey et al., 2013; Schwarz et al., 2012). Iba1 was used because it is specific to microglia, and its expression is constitutive in all microglia regardless of state (active or otherwise; Figure 1A).

2.2.2. Unbiased Stereology—Iba1-immunopositive microglia were counted using unbiased stereology as we have done previously (McCaffrey et al., 2013) using the optional fractionator probe of the Stereologer™ (Stereology Resource Center, Inc., MD) software on a Leica DM2500P scope (Leica Microsystems, Wetzlar, Germany). For each section (three per animal representing the anterior, mid-level, and posterior DG), the borders of the DG were traced at low magnification (5×) and then analyzed at high magnification (63×; Figure 1B). Final post-processing thickness was approximately 13 µm so the frame height was set to 10 µm with a guard height of 1 µm. Total frame area was 2500 µm² and frame spacing was set to 70 µm. Each of the three morphological categories of microglia in the postnatal brain were quantified: amoeboid (rounded and amorphous), ramified (extensive network of processes) and intermediate (Figure 1A; for a full description of each see (Karperien et al., 2013)).

2.3. Experiment 2: Adult Vole Microglia

Male and female prairie vole pups were orally exposed across PNDs 8–14, which has been identified as a crucial sociosexual developmental window in this species and akin to the neonatal period in rats/mice (Kramer et al., 2009). Animals received one of three doses of BPA: 5 µg/kg bw, 50 µg/kg bw (established reference dose) and 50 mg/kg bw (lowest observed adverse effect level). Doses were based upon average weight of pups on PND 8 and dissolved in 2.7g of Hydroxypropyl Beta CD – Pharm Grade in 10ml 0.9% NaCl. A fourth group received vehicle only. For all groups, delivery was 25 µl orally to the pups by micropipette (as described previously for mice (Palanza et al., 2002)). All litters contained at least one control and no more than one exposed animal per exposure per sex per litter.

Animals were sacrificed over PNDs 60–90. Subjects were given 0.05 ml buprenorphine via intra-peroneal injection, and then deeply anesthetized 15 min later with 0.05 ml of a ketamine-xylazine (at a concentration of 67.7 mg/kg and 13.33 mg/kg) mixture administered subcutaneously. Brains were removed and immersion fixed in 4% paraformaldehyde for 24 hrs at 4°C and transferred to fresh solution at 2 and 4 hrs post immersion. The brains were then cryoprotected in 30% buffered sucrose with 0.1% sodium azide and shipped to the Patisaul lab, where they were stored in fresh cryoprotectant overnight at 4°C then flash frozen, and stored at –80°C. Brains were coronally sectioned at 35 µm on a frozen sliding microtome. A prairie vole brain atlas is not available; thus the Paxinos and Watson rat brain atlas (Paxinos and Watson, 2007) was used to identify six consecutive sections per animal collectively containing the three regions of interest: the DG, amygdala, and primary somatosensory cortex. These sections were approximately equivalent to those selected from the rats in Experiment 1. Immunolabeling proceeded as described above. Because, to our knowledge, microglia have not previously been labeled in voles, to ensure the quality of the staining and optimize the dilution of the primary antibody (1:10,000), age-matched sections from 2 Wistar rats were included. Phenotypically, the labeling was identical. Only the sections with robust evidence of complete tissue penetration were used for the quantification and analysis.

Because, as expected with their more advanced age compared to the rats in Experiment 1, the vast majority of labeled vole microglia were of the ramified/stellate type (depicted in Figure 2A and B) only the total number of microglia was quantified. Labeled microglia were imaged using a QImaging Retiga 2000R 12-bit color camera (QImaging, Surrey, British Columbia, Canada) mounted on a Leica DM5000B scope (Leica Microsystems, Wetzlar, Germany) and quantified with the thresholding tool at 20× on the MCID Core Image software program (InterFocus Imaging Ltd., Cambridge, England). Three sections per animal comprising the DG were identified as described above for rats. For the amygdala (Bregma –2.76 through –3.36) three sections (representing the anterior, medial and posterior ends) were quantified per animal. For each section, three subregions were identified and counted using three counting frames of equal size placed medially to laterally (520 µm × 127 µm; depicted in Figure 3A). The most medial (Zone 1) approximately contained the medial amygdaloid nucleus (MeA) and the basolateral aspect of the central amygdaloid nucleus (CeA). Zone 2 contained the intra-amygdaloid division of the bed nucleus of the stria terminalis (STIA) and a portion of the posteroventral medial amygdaloid nucleus (MePV).

Zone 3 contained the medial aspects of the basolateral and basomedial amygdaloid nuclei (BLA and BMP respectively). For each region of interest, quantification was conducted at least twice (and those values averaged together to obtain the final data set) by observers blind to the exposure conditions.

2.4. Statistical Analysis

For all endpoints, exposure groups were analyzed (Sigmaplot 13) by two-way ANOVA with exposure and sex as factors. To maximize resolution regarding potential sex-specific effects, the data were then analyzed within sex, even if no main effect of sex was identified (noted for each endpoint). Significant main effects ($P \leq 0.05$) were then followed up with a protected Fisher's least significant differences (LSD) post-hoc test to evaluate pair-wise differences ($P \leq 0.05$).

3. Results

3.1. Postnatal Rat DG Microglia

The total number of microglia in the PND12 rat DG was not sexually dimorphic but significantly elevated by exposure ($F(1,40) = 5.44$; $P = 0.009$). In males, both EE ($P = 0.01$) and BPA ($P = 0.001$) increased the total number of microglia (Figure 1) but observed elevations in females did not reach statistical significance. Similarly, the number of amoeboid, ramified and intermediate microglia also did not differ by sex but exposure significantly affected two of the three populations. The number of amoeboid ($F(1,40) = 11.05$; $P = 0.001$) and thin ($F(1,40) = 10.88$; $P = 0.001$) microglia were significantly increased by exposure but no significant interaction with sex was detected (Figure 1). EE exposure approximately doubled the number of amoeboid microglia in both sexes ($P = 0.001$) while BPA increased numbers to a lesser extent and only significantly in males ($P = 0.05$). The number of intermediate microglia were significantly increased by EE ($P = 0.05$) and BPA ($P = 0.01$) in both sexes.

3.2. Adult Vole Microglia

As in the rat, immunolabeling was even and consistent across all sections and the microglia detectable in all regions of interest (Figure 2A and B). Nearly all of the microglia were in the stellate/ramified state.

3.2.1. Adult Vole DG—No main effects of exposure or sex were found for the total number of microglia in the adult vole DG but the interaction approached statistical significance ($F(1,108) = 2.18$; $P = 0.09$). Within females, there was a main effect of BPA exposure ($F(1,58) = 2.78$; $P = 0.05$) with higher numbers in the 50 mg BPA group compared to controls ($P = 0.05$). No effect of BPA was observed in males.

3.2.2. Adult Vole Amygdala—The amygdala was analyzed in three zones (Figure 3). The total number of microglia (across all three zones) did not differ by sex or exposure group. Among the females, the 50 mg BPA group had more microglia in Zone 1 than their unexposed conspecifics ($P = 0.04$), as did the 5 μ g BPA males ($P = 0.05$). No impact of sex or exposure was found for Zone 2. There was a significant interaction of sex and exposure

on the number of microglia in Zone 3 ($F(1,193) = 3.67$; $P = 0.01$) with the 5 μ g BPA males having more microglia in this area compared to same sex controls ($P = 0.02$).

3.2.3. Adult Vole Somatosensory Cortex—Total number of microglia did not differ by sex ($F(1,192) = 1.18$; $P = 0.3$) or exposure ($F(3,192) = 0.3$; $P = 0.8$) in the somatosensory cortex, nor was there any significant interaction ($F(3,192) = 0.3$; $P = 0.8$; data not shown).

Discussion

Perinatal exposure to EE or BPA approximately doubled the number of immature (amoeboid and intermediate) microglia in the PND 12 rat DG, an outcome which was more pronounced in males. The functional significance of this phenomenon and whether or not this colonizational change persists into adulthood, remains to be established, but prior work in our laboratory identified evidence of higher anxiety in their BPA-exposed siblings (Patisaul et al., 2012). BPA had less pronounced effects on the number of microglia in the adult vole DG. Increased numbers were only observed in females and only at the highest dose. Evidence for greater sensitivity to BPA was observed in the vole amygdala. Sub-region specific effects were detected in both sexes, with the lowest dose of BPA altering microglial numbers in the counting region containing the male BMP and portions of the BLA, the latter of which is a critical coordinator of anxiety. Collectively, these data emphasize the need to more comprehensively examine the age, sex and region-specific impact of hormone manipulation on microglial colonization, particularly in regard to potential species differences. That evidence of altered microglial numbers was seen in both species examined here supports our hypothesis that disruption of microglia may factor into the mechanisms by which environmental chemicals, including EDCs, impact the developing brain.

Prior work in rats has established that the colonization of the DG is sexually dimorphic during neonatal development, with males having more amoeboid (immature) microglia than females on PND 4 (Schwarz et al., 2012). This difference reverses by puberty onset, with females ultimately having more microglia with thick, long processes (mature morphology) than males at PND 30. That no sex difference in any microglia subtype was observed on PND 12 in the control group of the present study suggests that this reversal is in transition by the second week of life. Our data further suggest that estrogen and estrogenic endocrine disruptors, such as BPA, may either delay this transition (thereby maintaining high levels of amoeboid and intermediate microglia in the DG) or increase the numbers of immature microglia in this region. It remains unknown, however, if or how sexually dimorphic microglial colonization is differentially coordinated by sex hormones. It also remains to be determined through which estrogen receptor subtype the effects of EE and BPA reported here are mediated. Alternatively, these compounds could be acting via immunological or other “nonclassical” pathways to influence microglia colonization. We hypothesize that, in prepubertal females, endogenous estrogen, perhaps locally derived, stimulates the process by which a higher density of microglia with thick long processes arise in the maturing DG resulting in the sex differences reported on PND 30 (Schwarz et al., 2012). Ongoing studies in the laboratory are focused on resolving these fundamental questions.

Because, in the young adult rat, striking sex differences in the number of stellate microglia have been reported in the DG, amygdala, and parietal cortex (Schwarz et al., 2012), we hypothesized that we would see similar patterns in age-matched voles. That was not the case. As expected, the vast majority of microglia in the adult prairie vole brain were of the stellate/ramified phenotype, and their general distribution was similar to that of the rat, but no evidence of a sex difference in microglial numbers was found in any of the regions examined. The functional significance of this potentially important species difference is unclear but not entirely unexpected given the already well known species differences between rats and voles in social brain organization and behavior. Prairie voles are more affiliative than rats and display unique social characteristics more typical of humans including pair bonding, bi-parental care, and partner preference. These differences have been largely attributed to differences in oxytocin, vasopressin and estrogen receptor distribution, particularly in areas which ultimately feed into the mesolimbic dopamine system (Cushing and Wynne-Edwards, 2006; Lei et al., 2010; McGraw and Young, 2010; Ploskonka et al., 2015; Young et al., 2011). We have previously shown that some of these regions, including the paraventricular nucleus (PVN) and the bed nucleus of the stria terminalis (BNST) show sex-specific vulnerability to BPA (Sullivan et al., 2014). It is plausible that sex differences in microglial colonization and sensitivity to endogenous and exogenous hormones may be present in these and/or other regions and at different ages. Future studies will explore these possibilities (Lei et al., 2010).

While the impact of EE and BPA on the distribution and density of microglia in the PND12 rat DG was striking, and some evidence of BPA-related effects in voles was observed, how this altered composition may ultimately affect the function of these cells or manifest as changes in brain organization or behavior remains unclear. There is growing recognition that microglia play an important role in sexually dimorphic developmental processes including neuro/glia genesis, synaptogenesis and cell death, all of which have previously been shown to be affected by sex hormones (Forger et al., 2004; McCarthy, 2008; McCarthy et al., 2009; Sisk and Zehr, 2005) and, most critically for the present studies, developmental exposure to BPA (Kinch et al., 2015; MacLusky et al., 2005; Wolstenholme et al., 2011). Decades of neuropathology studies from ASD and schizophrenic patients have associated these disorders with defects in synapse formation/pruning particularly in subregions of the cortex and cerebellum (Arnold, 1999; Teffer and Semendeferi, 2012). Moreover, ASD patients have greater numbers of microglia in the cerebellum and cerebral cortex (Tetreault et al., 2012; Vargas et al., 2005), prompting speculation that altered microglial colonization and function may be contributory. It remains unknown, however, if their increased numbers are causal or a secondary symptom of underlying pathology. A separate but converging line of evidence has strongly associated elevated prenatal androgens with ASD risk (Alexander, 2014; Auyeung et al., 2009; Auyeung et al., 2010; Baron-Cohen, 2005; Baron-Cohen et al., 2005; James, 2014; Johnson et al., 2005; Knickmeyer et al., 2008; Kosidou et al., 2015; Palomba et al., 2012; Pohl et al., 2014) raising concerns that EDC exposure may also be contributory, but supporting evidence is extremely limited (Grandjean and Landrigan, 2014). Collectively our data support the hypothesis that sex hormones orchestrate the sex specific colonization of the social brain by microglia, and that disruption of this patterning is associated with heightened anxiety and impaired sociality in two species with different social characteristics.

Notably, while effects in males were limited, because the amygdala is known to play a critical role in the expression of prosocial behavior in male voles (Cushing et al., 2008), the changes observed in males at the lowest dose of BPA administered could meaningfully impact the formation of these behaviors.

That effects in the vole amygdala were observed at the lowest dose of BPA, and not dose dependent is consistent with the behavioral and neuroendocrine work we published previously from these animals (Sullivan et al., 2014). Females in the lowest dose group, but not the higher doses, displayed evidence of hyperactivity, and time spent socially investigating a novel animal was impaired in males at the middle dose. Loss of sex differences in dopaminergic neuron number in the PVN and BnST was also observed at the lowest dose of BPA (Sullivan et al., 2014). Why EDC effects are sometimes non-linear remains a critical and controversial question in the field but may be indicative of feedback systems, compensatory mechanisms, or other multi-system phenomenon (Kendig et al., 2010; Vandenberg et al., 2012).

In prior work we have shown that developmental exposure to BPA evokes hallmark features of compromised sociality including elevated anxiety and impaired social investigation in both rats and prairie voles (Patisaul and Bateman, 2008; Patisaul et al., 2012). Collectively, these data support the hypothesis that EDCs disrupt the social brain but a unified understanding of the chemical classes most likely to contribute is lacking, as are the mechanisms by which disruption occurs. Surprisingly few studies have specifically sought to establish which of the more than 85,000 chemicals that pervade our environment contribute to disorders of sociality, and their underlying neural features (Kalkbrenner et al., 2014; Miodovnik et al., 2011; Roberts et al., 2007). Associations between ASD traits and air pollution have been widely reported (Roberts et al., 2013; Volk et al., 2014) but a potential mechanism remains elusive. Studies in mice have demonstrated that prenatal stress can exacerbate air pollution induced neurocognitive deficits, particularly in males, via disruption of neuroinflammatory cascades (Bolton et al., 2013), implicating a role for microglia in psychosocial impairments (Bilbo, 2013). Elevated prenatal levels of trans-nonachlor (a component of the pesticide chlordane) and PBDE-28 (a fire retardant) have also been associated with compromised sociality, but not ASD specifically (Braun et al., 2014). Developmental exposure to phthalates (Miodovnik et al., 2011; Testa et al., 2012), the insecticide chlorpyrifos (de Cock et al., 2012), arsenic, manganese, BPA, BPS (Bisphenol S; the commercial replacement for BPA in can linings), numerous pesticides, fire retardants, and perfluorinated compounds have also been linked to neural effects (Grandjean and Landrigan, 2014; Kalkbrenner et al., 2014). Elevated gestational urinary concentrations of BPA have been correlated with adverse behavioral outcomes in children, including hyperactivity, anxiety, and executive function deficits (Braun et al., 2011; Braun et al., 2009; Harley et al., 2013; Miodovnik et al., 2011; Perera et al., 2012) suggesting that developmental BPA exposure impacts emotional development and social cognition. The mechanisms by which these effects occur remain largely elusive. Our data suggest that the disruption of microglia colonization could be contributory.

Conclusions

Using rats and prairie voles here we have shown for the first time that endocrine disruption can alter microglial colonization in brain regions fundamental to social behavior. We are also, to our knowledge, the first to explore the distribution of microglia in any area of the prairie vole brain. Our data suggest that there may be species differences in the sex-specific colonization of the social brain by microglia, and that early life exposure to estrogen or estrogenic EDCs could perturb this organization. The present studies used post-natal rats and adult voles because these tissues were available from prior work in our lab which documented BPA-related behavioral outcomes potentially attributable to disrupted microglial colonization and function. Future studies will focus on characterizing microglia colonization of the developing vole brain in greater detail, and explore how chemical exposures may alter their ontogeny to better understand how these glial cells may contribute to social deficits and disorders of the social brain.

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Highlights

- Microglia are the resident immune cells of the brain.
- Aspects of microglial colonization and function are sexually dimorphic.
- Little is known regarding the sex-specific colonization of brain regions critical for social behavior.
- We hypothesized that microglia colonization of the social brain could be altered by endocrine disruption.
- Two species were used, one which is promiscuous (rats) and one which is monogamous (prairie voles).
- Developmental exposure to the synthetic estrogen ethinyl estradiol (EE) or Bisphenol A (BPA) altered the sex-specific colonization of the social brain by microglia.

Figure 1.

Colonization of the PND 12 rat DG was not sexually dimorphic but influenced by BPA and EE exposure. (A) Microglia change shape across development. Prenatally, most are amoeboid in shape but by early adulthood obtain a highly branched, ramified morphology. Morphology is indicative of functional state with the amoeboid shape being indicative of activation in response to damage or injury, especially in adulthood. (B) The DG was identified using well established landmarks and the assistance of a brain atlas. (C) Total numbers of microglia did not differ by sex but a main effect of exposure was observed. Numbers were significantly increased by BPA and EE in males. (D) The number of amoeboid microglia were significantly increased by EE in both sexes. BPA had a lesser effect that only reached statistical significance in males. (E) Numbers of intermediate microglia were increased by EE and BPA in both sexes. (F) No effect of exposure was detected for number of ramified microglia. (Graphs depict means $\pm$ SEM; diagram in panel B adapted from (Paxinos and Watson, 2007); sample sizes per group shown in C; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

Figure 2.

Immunolabeling of microglia in the adult vole brain was consistent, robust and revealed no sex difference in microglial numbers in the DG. (A) Representative image depicting Iba1-immunoreactivity in the adult vole hippocampus including the DG. A higher magnification image of labeled microglia in the neighboring cortex is shown in (B) and shows that the vast majority displayed a highly branched morphology, indicative of the stellate type (see Figure 1). (C) Total number of DG microglia did not differ by sex but were significantly elevated in the females postnatally exposed to 50 mg/kg BPA. (Graphs depict means $\pm$ SEM; sample sizes per group shown in C; * $P < 0.05$)

Figure 3.

Postnatal BPA exposure had sex and region specific impacts within the adult vole amygdala.

(A) Microglia were quantified in three zones across three sections, the most medial of which is depicted here. (B) The total number of microglia in the amygdala (all zones and sections added together) did not differ by sex and was not altered by BPA. (C) In Zone 1, total

number of microglia were elevated in the 50 mg/kg BPA females and 5 µg/kg BPA males.

(D) No effects of BPA were observed in Zone 2 but (E) numbers were elevated in the 5

µg/kg BPA males in Zone 3. (Graphs depict means $\pm$ SEM; diagram in panel B adapted from (Paxinos and Watson, 2007); * $P < 0.05$; Abbreviations: medial amygdaloid nucleus posterodorsal portion (MePD), basomedial amygdaloid nucleus posterior portion (BMP), basolateral amygdaloid nucleus, anterior portion (BLA), central amygdaloid nucleus medial division (CeM), optic tract (opt).