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Perinatal programming of depressive-like behavior by inflammation in adult offspring mice whose mothers were fed polluted eels: Gender selective effects

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ABSTRACT

Several lines of evidence indicate that early-life inflammation may predispose to mental illness, including depression, in later-life. We investigated the impact of perinatal exposure to polluted eels on neonatal, postnatal, and adult brain inflammation, and on the resignation behavior of male and female adult offspring mice. The effects of maternal standard diet (laboratory food) were compared to the same diet enriched with low, intermediate, or highly polluted eels. Brain inflammatory markers including cytokines were assessed in offspring mice on the day of birth (i.e., on the postnatal day–PND 1), upon weaning (PND 21) and at adulthood (PND 100). Plasma myeloperoxidase and corticosterone levels were evaluated at PND 100. Immobility behavior of offspring was assessed in adulthood (i.e., at PNDs 95–100), using the tail suspension and forced swimming tests. Chronic brain inflammation was found in male and female offspring mice compared to controls, as assessed at PNDs 1, 21, and 100. The level of myeloperoxidase was found to be significantly higher in both adult males and females vs. control offspring. However, high corticosterone levels were only found in male offspring mice that were perinatally exposed to eels, suggesting a gender-selective dysregulation of the adult hypothalamic-pituitaryadrenal (HPA) axis. Gender-specific differences were also detected in adulthood in regard to offspring resignation behavior. Thus, compared to controls, males, but not females, whose mothers were fed eels during pregnancy and lactation exhibited a depressive-like behavior in adult age in both behavioral models of depression. Depressive symptoms were more pronounced in male mice perinatally exposed to either intermediate or highly polluted eels than those exposed to only lowly polluted eels. Our results indicate that early-life inflammatory insult is a plausible causative factor that induces the depressive phenotype exhibited by male adult offspring mice, most likely through a gender-specific HPA axis enhanced activation.

1. Introduction

During the perinatal period, the developing brain presents specific time windows in regard to both vulnerability and opportunity (Andersen, 2003; Arain et al., 2013; Hale et al., 2014; Ingber and Pohl, 2016; Marques et al., 2013; Viggiano, 2008). Several factors could interplay with these temporal windows, and the consequences may not necessarily be immediate, instead becoming manifest later in the life (Andersen, 2003; Hale et al., 2014; Spencer et al., 2007; Wainwright, 2002). Among the perinatal factors, the maternal life style, including dietary aspects, remains among the most important factors interfering with windows of vulnerability or opportunity in the immature brain (Fall, 2009; Hale et al., 2014; Lauritzen et al., 2001; Marques et al., 2013). Dependency of brain growth on the maternal supply of essential nutrients such as omega-3 fatty acids has already been emphasized (Dangour and Uauy, 2008; Lauritzen et al., 2001). Benefits of dietary ingredients following consumption of a healthy diet or supplementation on brain function have been reported, while negative effects due to dietary deficiencies have also been observed (Dangour and Uauy, 2008; Hale et al., 2014; Lauritzen et al., 2001; Wainwright, 2002). However, there is increasing evidence that our diet contains "good", i.e. physiologically supportive compounds promoting the development of young organism (e.g. vitamins, polyphenols, carotenoids, fatty acids) and "bad" ingredients, i.e. those inhibiting normal development (e.g. methylmercury, dioxins,

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pesticides, polychlorinated biphenyls – PCBs), many of which can act as neurotoxins and endocrine disruptors, as well as pro-inflammatory agents (Bohn et al., 2011; Dietert, 2012; Dridi et al., 2014; EFSA, 2005; Roegge and Schantz, 2006). Thus, the perinatal diet may act by altering or programming the development of the brain, e.g. by epigenetic regulation of inflammatory pathways such as hypomethylation of inflammatory genes (Bolton and Bilbo, 2014; Spencer, 2013). It has further been suggested that early-life exposure to pollutants including PCBs; dioxins; pesticides; polycyclic aromatic hydrocarbons (PAHs); and heavy metals, such as lead, arsenic, cadmium and mercury, can program misregulated inflammation in offspring throughout their life (Dietert, 2012). Surprisingly, developmental programming of brain and behavior by perinatal diet occurs more in males than in females, probably following the high vulnerability of the former to immune challenges that occur in early-life (Bolton and Bilbo, 2014). Perinatal programming by inflammation could result in decreased serotonin levels following exposure to elevated levels of pro-inflammatory cytokines (Bolton and Bilbo, 2014; Manuelpillai et al., 2005; Miller and Raison, 2016). An altered brain development trajectory may lead to modified behavior in later-life of the offspring, with a high risk of onset of diseases including neuropsychiatric disorders such as depression in adulthood (Andersen, 2003; Bilbo and Schwarz, 2009; Bolton and Bilbo, 2014). Studies of twins have revealed that the contribution of environmental factors in the pathogenesis of unipolar depression is very substantial, as genes only account for about 25–30% of the variance (Henn et al., 2004). Stress is considered to be the most important etiological factor of depression (Charney and Manji, 2004; Paykel, 2003). Undoubtedly, in addition to ongoing stress, early-life stress might also contribute to the onset of this mental illness throughout the individual's lifespan (Charney and Manji, 2004).

Fatty fish is a recommended weekly dietary component for pregnant women (Lichtenstein et al., 2006; SACN, 2004). This food item contains beneficial ingredients (e.g. omega-3 PUFAs and selenium) as well as detrimental ingredients (e.g. persistent organic pollutants – POPs – and metals) (Dridi et al., 2014; SACN, 2004). For instance, European eel (*Anguilla anguilla* L.) contains high concentrations of POPs, which are mainly of anthropic origin (Dridi et al., 2014; Macgregor et al., 2010; Szlinder-Richert et al., 2010). Eel is considered to be the most PCB-contaminated fish (EFSA, 2005; SACN, 2004). However, this fish is also a popular food item for many populations, e.g. in many European, Asian, and North America countries (Tsukamoto and Kuroki, 2014). Due to the long-standing human tradition of eel usage as food, several types of eel dishes are commonly consumed such as kabayaki, smoked eel, eel pie, jellied eels, angulas, eel stew, eel curry, and matelote d'anguille (Tsukamoto and Kuroki, 2014). Thus, in this study, we aimed to examine whether gestational and lactational exposure to eels naturally contaminated with pollutants including PCBs could induce changes in the resignation behavior of offspring male and female mice at adult age (i.e. at postnatal days – PNDs – from 95 to 100). For this purpose, the tail suspension and forced swimming tests, which are common behavioral tests to address depression, were used (Bouayed et al., 2012; Cryan et al., 2005; Zhao et al., 2008). We also performed an open-field test at PNDs 90–92, to evaluate locomotor activity in order to ensure that differences in activity levels did not bias the results in the other tasks (Bouayed et al., 2012).

Additionally, brain inflammation was assessed at PNDs 1, 21, and 100 by measuring levels of interleukin (IL)-1β, IL4, IL10, IL6, tumor necrosis factor alpha (TNFα), transforming growth factor beta (TGFβ), interferon gamma (IFNγ), and nitric oxide (NO). Moreover, the levels of the inflammatory enzyme myeloperoxidase (MPO) and the stress marker corticosterone were assessed in the plasma of adult offspring mice at PND 100.

2. Materials and methods

2.1. Animals

Forty pregnant CD1 mice (Charles River, France), obtained after a mating session performed in our laboratory as detailed in our previous studies (Bouayed et al., 2009; Dridi et al., 2014, 2016; Elnar et al., 2012), were used in this study. They were housed individually in standard cages with *ad libitum* access to water and food pellets (SDS Dietex, St Gratien, France) and they were maintained on a standard 12-h light/dark cycle (lights "on" starting at 8:00 p.m.), temperature controlled conditions (22 ± 2 °C), and a relative humidity of 55 ± 10%. The parturition day was considered to be postnatal day (PND) 1. All litters were randomly equalized to have n = 10 pups/litter, 5 pups/gender in order to prevent litter size bias. In regard to the remaining pups, only one pup/litter was randomly selected and sacrificed as described below in the section for Biochemical analyses. On PND 21 (i.e. at weaning), male and female offspring mice were separated from their mothers and housed in two different rooms to exclude effects of sexual pheromones on behavior at adult age. During the dark phase (1 h after lights "off") of the light/dark cycle, tests on animals were performed in a silent and isolated room, under dim red lighting for a maximum of 4 h per day of experimentation (i.e. experiments finished at 5 h after lights "off" to avoid circadian cycle bias). All animal procedures were carried out in accordance with the relevant European Union regulations (Directive 2010/63/EU), and they were approved by the institutional ethics committee of the University of Lorraine (authorization number CELMEA-2013-0010).

2.2. Perinatal diet manipulation

2.2.1. Eel matrix and pollutant quantification

A permit to fish for eels was obtained from the Ministry of the Walloon region, Belgium (authorization number DNF/DCP/CD705.1/Sortie 2007: 31416). Five river yellow eels (la Meuse, Belgium) were stunned and caught with a dip net in the spring of 2011. Additionally, 10 reared yellow eels were purchased from Zon-Aquafarming (Helmond, The Netherlands). Biologists from the University of Liège (ULg) identified both the river and the reared eels as *Anguilla anguilla* L. during the autopsy process. Eel muscle was then sampled, separated into two pools according to their origin, freeze-dried, mixed, and stored at −20 °C until paste preparation. Additionally, a portion of each pool was used to determine pollutant levels. PCBs and metals including mercury and lead were identified and quantified as previously described by Dridi et al. (2014). In this study, we also quantified the levels of other pollutants including polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (BDEs), and pesticides (see Table 1) in both the reared and the river muscle eels. Briefly, all of these organic pollutants were analyzed by gas chromatography (GC) coupled to high-resolution mass spectrometry (HRMS) using isotope dilution techniques for quantification (i.e., using ^{13}C labeled homologue compounds for PCDD/Fs, PCBs, and PAHs). Organochlorine pesticides were analyzed by GC connected with an electron capture detector (ECD). A full description of all analytical methods can be found elsewhere (Focant et al., 2001; Veyrand et al., 2007).

2.2.2. Eel paste preparation

The contaminated food (paste consisting of eels and chow) was prepared for a group of mothers (n = 10) by mixing 10 g of powdered food pellets (SDS Dietex, St Gratien, France) with 10 ml of water, 0.5 ml of sweet syrup, and 1 ml of corn oil and 320 mg of