Periconceptional alcohol exposure alters the mesolimbic reward pathway and behaviour in offspring

E.S. Dorey,[1] C.L. Cullen,[1,2] K.M. Mah,[1] D. Zanfirache,[1] B.S. Muhlhausler[3] and K.M. Moritz,[1] [1]School of Biomedical Sciences, The University of Queensland, St. Lucia, QLD 4072, Australia, [2]Menzies Institute for Medical Research, University of Tasmania, Hobart, TAS 7005, Australia and [3]School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, SA 5005, Australia.

Prenatal exposure to a maternal "junk food" diet or alcohol intake in late pregnancy results in altered development of the mesolimbic reward pathway in the offspring and increases their preference for high-fat food and alcohol respectively (Ong & Muhlhausler, 2011; Fabio *et al.*, 2015). Around 22.5% of women report consuming alcohol during the first month of gestation (Ethen *et al.*, 2009). This is during the periconceptional period, an increasingly recognised critical window for programming of adult onset disease. While maternal consumption of high-fat diets around conception has been associated with altered development of the reward pathway in adult offspring, the effects of periconceptional ethanol exposure on this pathway remain unknown (Grissom *et al.*, 2014). Therefore, in this study we aimed to investigate the effect of periconceptional alcohol exposure on food and alcohol preference and gene expression within the mesolimbic reward pathway in adult offspring.

Female Sprague Dawley rats were placed on a liquid control or 12.5% v/v ethanol diet (PC:EtOH, n=10-12/group) from 4 days before mating until embryonic day 4. Dams were allowed to litter down and offspring were weighed monthly. At 15 months(m) of age, rats underwent a high fat/cholesterol (22% Fat, 0.15%, SF00-219, Specialty Feeds, WA, HFD) food preference test. Rats were given free access to standard rat chow and HFD for a period of four days following a chow only period and their relative intake of each food type measured during this time. At 18m, rats underwent a 4 day two-bottle choice ethanol preference test (6% vol/vol EtOH or H2O). EtOH and water consumption was calculated (g/gBW). At 19m, offspring were killed (Lethobarb; 0.1ml/100g), abdominal girth (AG) was measured and fat pads (visceral, gonadal, subcutaneous and retroperitoneal) dissected out and weighed. Brains were excised, the ventral tegmental area (VTA) isolated and dissected before being snap frozen for subsequent analysis of mRNA levels of μ-opioid receptor (*Oprm1*), Dopamine receptor D1 (*Drd1*) and Dopamine receptor D2 (*Drd2*) using qPCR. Data obtained from male and female offspring were analysed separately.

PC:EtOH exposed male, but not female, offspring exhibited increased intake of HFD ($P<0.05$), particularly during days 3 and 4 of the food preference test when compared to their control counterparts. There was no effect of PC:EtOH on the intake of standard chow during the food preference test. PC:EtOH did not alter ethanol intake in either male or female offspring. However, PC:EtOH females showed increased total fluid consumption compared to controls ($P<0.05$) during the ethanol preference test. This was due to a significant increase in water, but not ethanol, consumption during the test period ($P<0.05$). PC:EtOH exposure did not alter body weight, AG or relative brain weight, in either male or female offspring, although relative visceral fat tended ($P=0.08$) to be increased in PC:EtOH males. PC:EtOH exposure increased relative mRNA expression of *Oprm1* and *Drd2* ($P<0.05$), but not *Drd1* in the VTA of female offspring. Interestingly, no alterations in expression were observed in male PC:EtOH offspring.

This study demonstrates that PC:EtOH can alter adult food preference in a sex dependent manner. In males, while animals of both groups initially ate similar amounts of HFD, the sustained consumption by PC:EtOH males indicates increased seeking of high-fat foods. This appears at least partially independent of general reward seeking behaviour as no alterations to EtOH consumption were apparent. Sexually dimorphic alterations in mRNA expression were also observed with increased expression of *Oprm1* and *Drd2* in PC:EtOH females only, despite no changes observed in preference for either HFD or EtOH. These results suggest that there may be other mechanisms contributing to the adult food preference seen in male offspring. Our study is the first to establish that PC:EtOH can change reward seeking behaviour in offspring and alter the mesolimbic reward pathway. These results highlight the importance of avoiding alcohol when planning a pregnancy.

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