Maternal cafeteria diet consumption and the programming of food preferences in the offspring: the role of the mu-opioid receptor

Jessica Rose Gugusheff

FoodPlus Research Centre

School of Agriculture, Food and Wine

The University of Adelaide

December 2014

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Numerous studies in rodent models have shown that the offspring of dams fed a high-fat high-sugar (cafeteria) diet throughout pregnancy and lactation develop a specific preference for the same kinds of foods in adulthood. Furthermore, studies into potential mechanisms have revealed that the offspring of cafeteria diet fed dams also have altered expression of key components of the mesolimbic reward pathway including the mu-opioid receptor. The current work used a rodent model to look specifically at the role of the mu-opioid receptor in the programming of food preferences and investigated when during development exposure to a maternal cafeteria-style diet could be most harmful.

The first aim of this thesis was to isolate whether exposure to a cafeteria diet before birth or in the pre-weaning period had a greater effect on the adult food preferences of the offspring. Using a cross-fostering method, we demonstrated that the male offspring of control or cafeteria diet fed (JF) dams that were cross-fostered at birth onto JF dams exhibited higher fat intake when challenged with a cafeteria diet at 7 weeks of age than offspring exposed to the cafeteria diet only before birth or not at all. Building on this work, we then investigated the effect of maternal cafeteria diet exposure on the postnatal development of the mu-opioid receptor. Using an in situ hybridisation method, we showed that female offspring of JF dams had reduced expression of the mu-opioid receptor in the ventral tegmental area in late postnatal development (week 3,4) relative to controls but not at the earlier timepoints explored (birth, week 1). The outcomes of the first two chapters of this thesis highlight the importance of the postnatal period in the establishment of offspring food preferences.

The final experiment, which forms the final two chapters of the thesis, used an opioid receptor antagonist to examine in greater detail the potential of the mu-opioid receptor as a mechanism for the programming of food preferences. We demonstrated that whilst the administration of the opioid receptor antagonist naloxone in the fourth week of life significantly reduced fat intake in control offspring given access to a cafeteria diet immediately postweaning, it failed to do so in male JF offspring and was less effective at reducing fat intake in JF females. This outcome provides evidence that changes in mu-opioid receptor expression induced by early life exposure to a cafeteria diet may indeed have functional consequences for the regulation of palatable food by the offspring. We also hypothesised that opioid receptor blockade during the fourth week of life would have long term effects on the food preferences of offspring; this however was not observed in the present study.

Nevertheless, this thesis provides considerable evidence to suggest that alterations in the development of the mu-opioid receptor plays an important role in the programming of food preference in offspring exposed to cafeteria diet in early life. In addition, it also identifies the postnatal period as potentially being 'critical window' during which exposure to cafeteria diet is most harmful to the offspring.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give my permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Jessica Rose Gugusheff
BSc. (Honours, First Class)
December 2014

ACKNOWLEDGMENTS

I would first and foremost like to acknowledge my primary supervisor Dr Beverly Muhlhausler, whose help, guidance and support throughout my PhD candidature has made this process possible. Thank you Bev for everything you have done, you have inspired me to grow both as a researcher and as a person and for that I will be forever grateful.

I would also like to acknowledge my co-supervisor Professor Robert Gibson as well as Dr John Carragher for their constructive criticism and review of my presentations and papers. Your efforts have not only helped me improve my presentation skills but also provided an invaluable alternate viewpoint that has enhanced the quality of my thesis. Thank you.

To Zhi Yi Ong- thank you for always being so generous with your time and answering all my questions, especially early on in my candidature. You really set the standard as to what a PhD student in our lab should be like and should be very proud of the wonderful researcher you have become. To Pamela Sim- thank you so much for everything, from the 6:30am morning conversations to the 7:30pm evening naloxone injections. I honestly don't think I would have got through this without you.

Also, I would like to thank the entire FoodPlus research group (especially those who assisted with the animal work) for all your help and camaraderie. Having a great lab group around me has made doing my PhD all the more enjoyable. I would particularly like acknowledge Dao Hunyh and Mini Vithayathil with whom I share an office.

In addition, I would like to acknowledge the financial support I have received from a Australian Postgraduate Award as well as Healthy Development Adelaide and the Women's and Children's Hospital Foundation.

Last but certainly not least; I would like to thank my family, for all their love, support and sacrifices, especially my mum, Sylvana and sister Tahlia. Thank you for always being there to share my laughter and help me through my tears. I would not be person I am today without you.

ABBREVIATIONS

ad libitum	to any desired extent
AgRP	agouti-related protein
AMPH	amphetamine
ANOVA	analysis of variance
ARC	arcuate nucleus
BMI	body mass index
BDNF	brain-derived neurotrophic factor
bp	basepairs
CART	cocaine amphetamine related transcript
Cdk5	cyclin-dependent kinase 5
cDNA	complementary deoxyribonucleic acid
CO_2	carbon dioxide
CoV	coefficient of variance
CRF	corticotrophin releasing factor
D1	dopamine 1 receptor
D2	dopamine 2 receptor
DA	dopamine
DARPP-32	dopamine- and cAMP-regulated neuronal phosphoprotein-32
DAT	dopamine active transporter
E	embryonic day
ELISA	enzyme linked immunosorbent assay
ENK	enkephalin
GABA	gamma-aminobutyric acid
IRS-1	insulin receptor substrate-1
JF	junk food
L-DOPA	L-dihydroxyphenylalanine
LH	lateral hypothalamus
LPL	lipoprotein lipase
mRNA	messenger ribonucleic acid
MSN	medium spiny neuron

NAc	nucleus accumbens
NEFA	non-esterified fatty acid
NPY	neuropeptide Y
ob/ob	obese
PBN	parabrachial nucleus
PENK	proenkephalin
PFC	prefrontal cortex
PI3K	phosphoinositide 3-kinase
PND	postnatal day
POMC	pro-opiomelanocortin
PPARγ	peroxisome proliferator activated receptor γ
PVN	paraventricular nucleus
PW	postnatal week
qRT-PCR	quantitative reverse transcription real time polymerase chain reaction
RNA	ribonucleic acid
SEM	standard error of the mean
SPSS	statistical package for social sciences
TH	tyrosine hydroxylase
VTA	ventral tegmental area
WHO	World Health Organisation

PUBLICATIONS ARISING FROM THIS THESIS

1. **Gugusheff, JR**., Ong, ZY., & Muhlhausler, BS. (2014). The early origins of food preferences: targeting the critical windows of development. *The FASEB Journal* In Press

2. **Gugusheff, JR**., Ong, ZY., & Muhlhausler, BS. (2014). Naloxone treatment alters gene expression in the mesolimbic reward system in 'junk food'exposed offspring in a sex-specific manner but does not affect food preferences in adulthood. *Physiology & Behaviour 133* 14-21.

3. **Gugusheff, JR**., Vithayathil, M., Ong, ZY., & Muhlhausler, B. S. (2013). The effects of prenatal exposure to a 'junk food'diet on offspring food preferences and fat deposition can be mitigated by improved nutrition during lactation. *Journal of Developmental Origins of Health and Disease*, *4*(05), 348-357.

4. **Gugusheff JR**, Ong, ZY, Muhlhausler, BS (2013). A maternal "junk-food" diet reduces sensitivity to the opioid antagonist naloxone in offspring postweaning. *The FASEB Journal*, *27*(3), 1275-1284.

5. Muhlhausler BS, **Gugusheff JR**, Ong ZY, et al. (2013) Nutritional approaches to breaking the intergenerational cycle of obesity *Canadian Journal of Physiology Pharmacology* **91**, 421-8.

6. Muhlhausler, BS., **Gugusheff, JR**., Ong, ZY., & Vithayathil, M. A. (2013). Pregnancy, obesity and insulin resistance: maternal overnutrition and the target windows of fetal development. *Hormone Molecular Biology and Clinical Investigation*, *15*(1), 25-36.

7. Ong, ZY, **Gugusheff JR**, & Muhlhausler BS. (2012). Perinatal overnutrition and the programming of food preferences: pathways and mechanisms. *Journal of Developmental Origins of Health and Disease*, *1*(1), 1-10.