

2012

Alterations in Hypothalamic and Brainstem Neurotransmitter Signalling Associated with Olanzapine-Induced Metabolic Side-Effects

Katrina Green

University of Wollongong

Recommended Citation

Green, Katrina, Alterations in Hypothalamic and Brainstem Neurotransmitter Signalling Associated with Olanzapine-Induced Metabolic Side-Effects, Doctor of Philosophy thesis, School of Health Sciences, University of Wollongong, 2012.
<http://ro.uow.edu.au/theses/3579>

UNIVERSITY OF WOLLONGONG

COPYRIGHT WARNING

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site. You are reminded of the following:

Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material. Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

Alterations in Hypothalamic and Brainstem Neurotransmitter Signalling Associated with Olanzapine-Induced Metabolic Side-Effects

A thesis submitted in (partial) fulfilment of the
requirements for the award of the degree

DOCTOR OF PHILOSOPHY

From

University of Wollongong
School of Health Sciences

By

Katrina Green, BSc(Biol/BioMed Sc) (Hons)

2012

Certification

I, Katrina L. Green, declare that this thesis, submitted in (partial) fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Health Sciences, University of Wollongong, is entirely my own work unless otherwise referenced or acknowledged. This manuscript has not been submitted for qualification at any other academic institution.

Katrina Green

2012

Statements

In accordance with the University of Wollongong thesis committee 'Guidelines for Higher Degree Research Candidates on the Preparation and Submission of Higher Degree Research Theses' (January 2011) this PhD is presented in 'Journal Article Style'. It is comprised of a series of three original studies published in peer-reviewed journals, of which I am the first author. I hereby declare that I am the primary designer of these studies, have carried out experimental procedures, data analysis and manuscript preparation.

Katrina Green

2012

I consent to the presentation of this PhD in 'Journal Article Style' and I acknowledge the above statement pertaining to student contribution to be correct.

Assoc. Prof. Chao Deng, Supervisor

2012

Prof. Xu Feng Huang, Supervisor

2012

Publications

The following publications and presentations have arisen directly from work contained within this thesis.

Publications in Refereed Journals:

- **Weston-Green K, Huang X-F, Deng C.** Alterations to melanocortinerbic, GABAergic and cannabinoid neurotransmission associated with olanzapine-induced weight gain. *PLoS One*, 7(3): e33548 –March 16, 2012
- **Weston-Green K, Huang XF, Lian J, Deng C (2011).** Effects of olanzapine on muscarinic M3 receptor binding density in the brain relates to weight gain, plasma insulin and metabolic hormone levels. *European Neuropsychopharmacology*. 22(5): 364-373
- **Weston-Green K, Huang X-F, Deng C (2011).** Olanzapine treatment and metabolic dysfunction: a dose response study in female Sprague Dawley rats. *Behavioural Brain Research*. 217(2): 337-346
- **Weston-Green K, Huang X-F, Deng C (2010).** Sensitivity of the female rat to olanzapine-induced weight gain--Far from the clinic? *Schizophrenia Research* 116(2-3): 299-300

Published Abstracts:

- **Weston-Green K, Huang X, Deng C, (2010).** Olanzapine decreases POMC mRNA expression and M3 receptor binding density in the hypothalamus and brainstem: Pathway for weight gain side-effect? 11th Australasian Schizophrenia Conference (ASC2010), September 2010, Sydney, Australia. *Australian and New Zealand Journal of Psychiatry*, 44(Supp. 1):A55
- **Weston-Green K, Huang X, Kang K, Deng C, (2010).** Olanzapine decreases cannabinoid CB1 receptors in the hypothalamus and brainstem, possibly through muscarinic M3 receptor antagonism. *Collegium Internationale Neuro-Psychopharmacologicum XXVII World*

Congress, Hong Kong, China, June 2010. *International Journal of Neuropsychopharmacology* 13(Supp. 1):111-112

- Deng C, **Weston-Green K**, Huang X (2007). Dose-dependent effects of atypical antipsychotic drug olanzapine on weight gain and cholecystokinin levels in rats. *7th Biennial Meeting of the Chinese Society for Neuroscience*, 24-28 Oct 2007, Hangzhou, China. *Neuroscience Bulletin*, 23(Supp. 1): 352-352

Oral Conference Presentations:

- **Weston-Green K**, Huang X, Deng C, (2011). Neuroendocrinological regulation of insulin dysfunction induced by olanzapine. *Australian Neuroscience Society 31st Annual Meeting*, Jan-Feb 2011, Auckland, New Zealand, Oral-13
- **Weston-Green K**, Huang X, Lian J, Deng C, (2010). Olanzapine-induced metabolic dysfunction: a role for central muscarinic M3 receptors. *Australian Neuroscience Society 30th Annual Meeting in conjunction with The Australian Physiological Society 50th Anniversary Meeting (ANS/AuPS 2010)*, Jan-Feb 2010, Sydney, Australia, Oral-03

Conference Proceedings:

- **Weston-Green K**, Huang X, Deng C, (2010). The dosage response to the side-effect of metabolic dysfunction in female rats following olanzapine treatment. *Australian Neuroscience Society 30th Annual Meeting in conjunction with The Australian Physiological Society 50th Anniversary Meeting (ANS/AuPS 2010)*, Jan-Feb 2010, Sydney, Australia, p-63
- **Weston-Green K**, Deng C, Han M, Huang (2007). Effects of antipsychotics on cannabinoid receptors and satiety hormones: Mechanisms for the side-effect of weight gain. *Higher Degree Research Student Conference*, Sept 2007, University of Wollongong, Wollongong, Australia

Additional Publications:

The following publications and presentations have arisen as a result of other projects I have been involved in during my doctoral studies:

Publications in Refereed Journals:

- Deng C, **Weston-Green K**, Huang X-F (2010). The role of histaminergic H1 and H3 receptors in food intake: A mechanism for atypical antipsychotic-induced weight gain? *Progress in Neuropsychopharmacology and Biological Psychiatry* 34(1): 1-4
- **Weston-Green K**, Huang X-F, Han M, Deng C (2008). The effects of antipsychotics on the density of cannabinoid receptors in the dorsal vagal complex of rats: implications for olanzapine-induced weight gain. *The International Journal of Neuropsychopharmacology* 11(06): 827-835

Conference Proceedings:

- Lian J, **Weston-Green K**, Kang K, Huang X, Deng C, (2010). Olanzapine decreases [³H]-SR141716A binding to cannabinoid CB1 receptors in the rat hippocampus and auditory cortex: a dosage-dependent response. *Australian Neuroscience Society 30th Annual Meeting in conjunction with The Australian Physiological Society 50th Anniversary Meeting (ANS/AuPS 2010)*, Jan-Feb 2010, Sydney, Australia, p62
- **Weston-Green K**, Deng C, Han M, Huang X (2007). Effects of antipsychotic drugs on weight gain and CB1 receptors in the dorsal vagal complex of rats. 7th IBRO World Congress of Neuroscience, Melbourne, Australia. p144

Table of Contents

Certification	ii
Statements	iii
Publications	iv
Table of Contents	vii
List of Figures	x
List of Tables	xi
List of Abbreviations	xii
Abstract	xiii
Acknowledgements	xvii
Chapter One	1
1.1. Introduction	1
1.2. Review of Literature	2
1.2.1 Schizophrenia	2
1.2.2. Antipsychotic Drugs: A History and Dichotomy of Generations	3
A. First Generation 'Typical' Antipsychotic Drugs	3
B. Second Generation 'Atypical' Antipsychotic Drugs	5
1.2.3 The Control of Energy Homeostasis	9
A. Metabolic Hormonal Signals and the Effect of Antipsychotic Drugs	9
I. Insulin	10
II. Ghrelin	12
III. Cholecystokinin (CCK)	13
IV. Peptide Tyrosine Tyrosine (PYY)	14
V. Leptin	15
B. The Brain-Gut Axis	16
C. The Neural Control of Appetite and Glucose Homeostasis	18
I. The Role of the Hypothalamus and Caudal Brainstem in Energy Homeostasis	18
Pro-opiomelanocortin (POMC) and Neuropeptide Y (NPY)	20
II. The Central Control of Insulin Secretion and Glucose Homeostasis	22

The Cephalic and Gastric Phases of Insulin Secretion	23
III. Neurotransmitter Signalling Pathways in Metabolism.....	24
a. Cholinergic Muscarinic Neurotransmission: Role in Glucose and Energy Homeostasis, and Antipsychotic-Induced Metabolic Dysfunction.....	25
i. Parasympathetic and Sympathetic Regulation of Insulin Secretion.....	25
ii. The Muscarinic M3 Receptor.....	26
iii. M3 Receptor Role in Antipsychotic-Induced Metabolic Dysfunction	27
b. Cannabinoid System: Role in Food Intake and Antipsychotic-Induced Weight Gain	31
i. Cannabinoid Neurotransmission and Antipsychotic Drugs: Role in Metabolic Dysfunction	35
1.2.4 Animal Model of Antipsychotic-Induced Weight Gain	36
A. Antipsychotic Drug Selection, Dosage Regime and Administration Method	36
B. Gender Differences.....	40
C. Overall Comments on Animal Models of Antipsychotic-Induced Weight Gain	42
1.3 Aims.....	43
1.3.1 General Aim	43
1.3.2 Specific Aims	43
1.3.3 Hypotheses	44
1.3.4 Significance.....	44
1.4 General Methods	46
1.4.1 Ethics Statement.....	46
1.4.2 Animals and Drug Treatment.....	46
1.4.3 Behavioural Analysis: Open Field Testing.....	47
1.4.4 Hormonal and Adipose Measurements	47
1.4.5 Histology	49
1.4.6 Receptor Binding	49
1.4.7 <i>In-situ</i> Hybridisation	51
1.4.8 Statistical Analysis.....	51
1.5 Thesis Summary.....	53
1.5.1 Olanzapine Treatment and Metabolic Dysfunction: A Dose Response Study in Female Sprague Dawley Rats	54
1.5.2 Effects of olanzapine on muscarinic M3 receptor binding density in the brain relates to weight gain, plasma insulin and metabolic hormone levels	55
1.5.3 Alterations to Melanocortinerbic, GABAergic and Cannabinoid Neurotransmission Associated with Olanzapine-Induced Weight Gain	56

1.5.4 Summary Conclusion	57
Chapter Two	58
Olanzapine Treatment and Metabolic Dysfunction: A Dose Response Study in Female Sprague Dawley Rats	58
Chapter Three	69
Effects of Olanzapine on Muscarinic M3 Receptor Binding Density in the Brain Relates to Weight Gain, Plasma Insulin and Metabolic Hormone Levels	69
Chapter Four	80
Alterations to Melanocortinerbic, GABAergic and Cannabinoid Neurotransmission Associated with Olanzapine-Induced Weight Gain	80
Chapter Five	93
5.1 Overall Discussion and Conclusions	93
5.1.1 The Mechanisms of Olanzapine-Induced Metabolic Dysfunction	93
5.1.2. Recommendations for Further Research	101
5.1.3. Conclusion	104
References	106
Appendix One	133
A1.1 Sensitivity of the Female Rat to Olanzapine-Induced Weight Gain – Far From the Clinic?	133

List of Figures

Figure 1.1: Schematic of the Brain-Gut Axis.	17
Figure 1.2: Potential Mechanism for the Involvement of Muscarinic M3 Receptors (M3Rs) in Olanzapine-Induced Metabolic Dysfunction	29
Figure 1.3: Schematic of Cannabinoid-Mediated Retrograde Transmission	33
Figure 1.4: Example of Open-Field Testing and Ethovision Trace.....	48
Figure 1.5: Schematic of the rat brain.....	50
Figure 5.1: A Potential Mechanism Underlying Olanzapine-Induced Metabolic Dysfunction Based on this Study	99-100

List of Tables

Table 1.1: Receptor binding affinities of selected antipsychotic drugs (K_i nM)	7
Table 5.1: Summary of Main Findings in Chapters 2-4	94

List of Abbreviations

Δ^9 -THC	Delta-9-Tetrahydrocannabinol
α -MSH	Alpha-Melanocyte Stimulating Hormone
2-AG	2-Arachidonoyl Glycerol
ACh	Acetylcholine
AgRP	Agouti-Related Peptide
AP	Area Postrema
Arc	Arcuate Nucleus
BMI	Body Mass Index
CART	Cocaine- and Amphetamine-Related Transcript
CB1R	Cannabinoid CB1 Receptor
CB2R	Cannabinoid CB2 Receptor
CBR	Cannabinoid Receptor
CCK	Cholecystokinin
CCK1R (CCK-A)	Cholecystokinin-1 Receptor (formerly CCK-A)
CCK2R (CCK-B)	Cholecystokinin-2 Receptor (formerly CCK-B)
DMN	Dorsal Motor Nucleus
DVC	Dorsal Vagal Complex
EPS	Extrapyramidal Side-Effects
GABA	γ -Aminobutyric acid
GAD	Glutamic Acid Decarboxylase
GAD ₆₅	Glutamic Acid Decarboxylase Isoform 65
GAD ₆₇	Glutamic Acid Decarboxylase Isoform 67
GHS-R	Growth Hormone Secretagogue Receptor
GI	Gastrointestinal
GPCR	G-Protein-Coupled Receptor
LHA	Lateral Hypothalamic Area
M3R	Muscarinic M3 Receptor
MC4-R	Melanocortin-4 Receptor
mRNA	Messenger Ribonucleic Acid
NPY	Neuropeptide Y
NTS	Nucleus of the Solitary Tract
POMC	Pro-opiomelanocortin
PVN	Paraventricular Nucleus
PYY	Peptide Tyrosine Tyrosine
PYY ₍₃₋₃₆₎	Peptide Tyrosine Tyrosine ₍₃₋₃₆₎
SD	Sprague Dawley
SGAs	Second Generation Antipsychotic Drugs
TID	Three Times Daily
VMH	Ventromedial Hypothalamic Nucleus

Abstract

Second generation antipsychotics (SGAs) are a key pharmacotherapy for the treatment of schizophrenia but can cause serious metabolic side-effects, including obesity and type II diabetes mellitus. Clinical studies have associated the high risk SGA olanzapine with hyperphagia, increased abdominal adiposity, reduced physical activity and altered circulating metabolic hormone levels, i.e. leptin, ghrelin and insulin; however, the underlying mechanisms remain unclear. The hypothalamus and caudal brainstem are well-documented for their involvement in appetite and energy homeostasis. Altered neurotransmission in these regions during olanzapine treatment may contribute to metabolic dysfunction, however, the ability to examine drug effects on neural signalling in humans *in-vivo* is limited. Therefore, an animal model is required that mimics numerous aspects of clinical metabolic side-effects caused by olanzapine treatment. Previous pre-clinical studies have utilised a range of olanzapine dosages and treatment regimes, however the dose that best resembles the clinic is unclear.

Chapter 2 aimed to test the validity of the female rat model through its ability to mimic clinical olanzapine-induced metabolic dysfunction. Female Sprague Dawley rats were treated orally, three times daily with olanzapine (0.25mg/kg, 0.5 mg/kg, 1.0 mg/kg, 2.0mg/kg), self-administered in a sweet cookie dough pellet at 8-hourly intervals or vehicle (n=12/group) for 14-days. The dosage response in each parameter was examined in order to identify the most appropriate dose to use in the rat model. Olanzapine increased body weight (0.5, 1.0, and 2.0mg/kg olanzapine treatment groups), food intake (2.0mg/kg olanzapine) and feeding efficiency (0.5-2.0mg/kg olanzapine), but did not affect water intake. Subcutaneous inguinal (1.0-2.0mg/kg olanzapine) and intra-abdominal perirenal fat were increased (2.0mg/kg), and a trend for an increase in periovary fat mass was observed in the 2.0mg/kg olanzapine treatment group ($p=0.07$). Interscapula brown adipose tissue mass was unchanged. Olanzapine decreased insulin in all

dosage groups (0.25mg/kg-2.0mg/kg), increased circulating ghrelin and CCK, but had no effect on peptide YY₍₃₋₃₆₎. Locomotor activity in the open field arena was reduced following olanzapine treatment (0.5-2.0mg/kg olanzapine). Together, these findings demonstrate that multiple aspects of clinically-reported metabolic side-effects associated with olanzapine treatment can be modelled in the rat. In addition, this chapter demonstrated that low, clinically-relevant dosages of olanzapine can cause metabolic effects when administered in-line with the half life of the drug (i.e. 8-hourly). Therefore, this model is a valid foundation that can give insight into the *in-vivo* effects of olanzapine on neurotransmission in the brain.

Following the demonstration in Chapter 2 of the metabolic side-effects of olanzapine, Chapters 3 and 4 aimed to elucidate the mechanisms underlying olanzapine-induced weight gain and insulin dysregulation by examining the central *in-vivo* effects of olanzapine on key neurotransmitter signals that regulate metabolic homeostasis in the hypothalamus and brainstem; including the muscarinic, melanocortinerbic, GABAergic and cannabinoid systems. In Chapter 3, the dosage effects of olanzapine on muscarinic M3 receptor (M3R) binding density in the hypothalamic arcuate (Arc) and ventromedial hypothalamic nucleus (VMH), and the dorsal vagal complex (DVC) of the caudal brainstem were investigated, and relationships between changes in M3R density and metabolic hormone levels were examined. Olanzapine increased M3R binding density in the Arc and DVC (0.5-2.0mg/kg olanzapine), and the VMH (0.25-2.0mg/kg olanzapine), and decreased blood glucose levels. Changes in M3R binding density significantly correlated with plasma insulin, ghrelin and CCK, as well as food intake and body weight. Olanzapine is a potent M3R antagonist, therefore the increase in M3R density was likely to be a compensatory upregulation. Inhibition of the cholinergic pathway for insulin secretion by olanzapine's M3R blockade may explain the hypoinsulinaemia observed in treated rats, an effect that was irrespective of dosage. The data shows that M3R blockade by olanzapine was also associated

with altered levels of ghrelin and CCK. The results show for the first time that olanzapine acts on M3Rs in regions of the brain that regulate appetite and insulin secretion, and support a role for M3Rs in modulating insulin, ghrelin and CCK during olanzapine treatment, possibly via cholinergic vagal innervation of the GI tract. This study provides a novel mechanism for olanzapine's diabetogenic and weight gain liability that can also apply independent of obesity; as olanzapine can promote the onset of diabetes in normal-weight individuals in the clinic.

Several other key neurotransmitter systems involved in energy homeostasis may also play a role in olanzapine-induced metabolic side-effects, including the potent orexigen neuropeptide Y (NPY) and anorexigenic pro-opiomelanocortin (POMC) of the melanocortinergic system. The GABAergic and endogenous cannabinoid systems are also documented for their effects on appetite and form part of the hypothalamic microcircuitry that regulates POMC and NPY neurotransmission. Chapter 4 revealed an olanzapine-induced increase in NPY (1.0-2.0mg/kg olanzapine) and reduction in POMC mRNA expression (0.5-2.0mg/kg olanzapine) in the Arc but not in the DVC. Cannabinoid CB1 receptor (CB1R) binding density decreased (Arc: 0.25-2.0mg/kg olanzapine, DVC: 0.5-2.0mg/kg olanzapine) and GAD₆₅ mRNA expression increased (Arc and DVC: 1.0-2.0mg/kg olanzapine) in both the Arc and DVC during olanzapine treatment. Taken together, these results demonstrate that olanzapine alters the balance of major neuronal regulators of energy homeostasis in a dose-sensitive manner that favours body weight gain. These data provide evidence to support a novel mechanism for olanzapine-induced weight gain, whereby increased NPY and enhanced inhibitory GABAergic output, through reduced CB1R density, contribute to POMC inhibition.

Collectively, this thesis is novel in revealing that changes to major neurotransmission systems involved in controlling energy homeostasis in the hypothalamus, and to a lesser extent, the

brainstem, contribute to olanzapine-induced metabolic dysfunction. High expression of orexigenic NPY and GABA, together with low expression of anorexigenic POMC and CB1R density may favour positive energy balance during olanzapine treatment. The data also suggests that blockade of the M3R in the hypothalamus and brainstem by olanzapine contributes to its diabetogenic liability by causing insulin dysregulation, and to its weight gain risk by altering other metabolic hormones including ghrelin and CCK levels, possibly via disruption to vagal cholinergic innervation of the GI tract. Together, metabolic hormone imbalance, due to M3R antagonism by olanzapine, may lead to alterations in neurotransmission through interactions between the brain-gut axis that regulates energy homeostasis. In addition, this thesis reveals that a dosage of 1.0mg/kg olanzapine (t.i.d.) is sufficient to induce changes in most parameters measured. Overall, this thesis presents considerations for the discovery of new antipsychotic drugs with low risk of metabolic side-effects and provides direction for the experimental design of future animal modelling studies of antipsychotic effects.

Acknowledgements

I wish to thank the School of Health Sciences and the Illawarra Health and Medical Research Institute, University of Wollongong (UOW), as well as the Schizophrenia Research Institute for their funding and support. I also benefited from conference travel grants from the Australian Neuroscience Society and from the Faculty of Health and Behavioural Sciences, UOW.

My sincere thanks to my supervisors, A/Prof Chao Deng and Prof Xu Feng Huang for their knowledge, dedicated support, inspiration, encouragement, and their kindness and understanding during my two (yes two) maternity leaves. My heartfelt thanks to Chao for helping me to build my knowledge to what it is today and for not letting me quit when things seemed too difficult. I feel very honoured and fortunate to have completed my honours and PhD years with such knowledgeable, inspirational and supportive academics. I would like to thank my friends and colleagues at the Centre for Translational Neuroscience for their encouragement and support. I also extend my gratitude to Ms Kelly Liu, Dr Kai Kang, Dr Mei Han, Ms Jiamei Lian, Dr Yinghua Yu, Ms Brittney Merryweather and Ms Gita Rahardjo for their expert technical assistance. My thanks to Dr Tracey Maddocks for her caring support during my animal experiments.

I wish to express my love and gratitude to my friends and family. Thanks to my brothers, Glenn and Phil, my dad Christopher, my parents-in-law Robyn and Bob, and sisters Katarina, Jodie and family for their positive attitudes and support. I thank my three little men, Hayden, Ollie and Ned - the reasons for which my world turns – thank you for your smiles, thank you for your cuddles. Words cannot express my gratitude and love for my husband Michael and for my mum, Silvia, for their selfless sacrifice in helping me to achieve this dream - this PhD has three authors because without you both it would never have been completed. Thank you, Michael, for believing in me, for playing both Mum and Dad during this time, for your support, and for encouraging me to go back to Uni in the first place. My final thanks to God for making this journey a part of His plan.

CHAPTER ONE

1.1. INTRODUCTION

Schizophrenia is a complex psychotic disorder that affects approximately 1% of the world's population and ranks within the top 10 causes of disability in developed countries world-wide. Individuals with schizophrenia have a mortality rate 2.5 times higher than the general population, and average life expectancy is reduced by approximately 10-years (Tandon et al., 2009). Antipsychotic drugs are a key pharmacotherapy for the treatment of schizophrenia and a number of other psychiatric illnesses. The newer 'second generation' antipsychotics (SGAs) are prescribed to treat a range of symptoms and lack the debilitating extra-pyramidal and motor dysfunction side-effects that are associated with older 'first generation' compounds. However, SGAs can induce metabolic disturbances such as weight gain, abdominal adiposity, dyslipidaemia, gluco-regulatory abnormalities and insulin-resistance (Newcomer, 2005), which are particularly distinct in individuals treated with the high risk drugs, olanzapine and clozapine (Allison et al., 2009; Newcomer, 2005). SGA-induced metabolic side-effects are of growing concern due to associated co-morbidities such as obesity, type II diabetes mellitus and cardiovascular disease. In addition, the incidence of diabetes and obesity in schizophrenia is 1.5-2.0 times higher than the general population (American Diabetes Association et al., 2004). These factors, combined with increasing SGA prescription in both adults and children (Hollingworth et al., 2010; Vitiello et al., 2009; Zuddas et al., 2011), highlight the urgency of the need to understand the mechanisms underlying SGA-induced metabolic side-effects. Numerous hypotheses have arisen over the past decade (Coccurello and Moles, 2010; Reynolds and Kirk, 2010), however, an indisputable answer has not yet been found. It is likely that a number of metabolic facets interplay in the pathogenesis of SGA-induced metabolic side-effects, due to their

broad receptor binding profile that allows multiple effects on central and peripheral cellular pathways. In order to examine these side-effects *in-vivo*, there is a need for a valid and reliable pre-clinical model of antipsychotic-induced metabolic dysfunction that closely resembles the clinic. Therefore, the present PhD focused on the effects of olanzapine, a SGA with a high metabolic liability, on key metabolic hormones and neurotransmitter systems in an animal model of olanzapine treatment. Overall, this series of studies aimed to understand the mechanisms underlying olanzapine-induced body weight gain and insulin dysfunction. A better understanding of these mechanisms may assist the design of a new generation of antipsychotic drugs that possess both therapeutic efficacy and reduced obesity / diabetes side-effects.

1.2. REVIEW OF LITERATURE

1.2.1 Schizophrenia

Schizophrenia is a mental disorder with diverse symptoms including retreat from reality, distorted thoughts, cognitive and motor impairment, emotional dysfunction and a decline in communication skills leading to social isolation, occupational disability and physical deterioration. Recent theory suggests that schizophrenia is best described as a conglomeration of syndromes and diseases rather than a single pathological state, due to the range of symptoms that can differ between individuals (Tandon et al., 2009). Broadly, schizophrenia consists of three symptom domains: positive, negative and cognitive. Positive symptoms include behavioural abnormalities such as speech and thought disorder, delusions and hallucinations, while the negative domain encompasses a decline in response such as flattened emotional expression, alogia, avolition-apathy and anhedonism (Andreasen et al., 1995; Tandon et al., 2009). Cognitive deficits include reduced executive function such as organisation, memory and attention deficits, and altered perception eg misinterpretation of behaviour and intent of others (Tandon et al., 2009).

Several methods can be used to treat or attenuate the symptoms of schizophrenia, including psychosocial rehabilitation and psychotherapy (Kern et al., 2009; Tandon et al., 2010); however pharmacological intervention through the use of antipsychotic drugs remains a key component of schizophrenia treatment. Unfortunately, the search for the perfect antipsychotic appears to be frustrated by the complexity of the disease, as a percentage of patients remain unresponsive to antipsychotic treatment (Meltzer, 1992; Suzuki et al., 2008). In addition, pharmacological therapy for schizophrenia treatment involves a life-long adherence to drugs that are associated with an array of side-effects (Anderman and Griffith, 1977; Bobes et al., 2003; Fakhoury et al., 2001; Newcomer, 2005; Poyraz et al., 2008; Voruganti et al., 2000; Wirshing et al., 2002). Therefore, despite robust improvements in psychopharmacological therapy over the past 60-years, more research is required to aid discovery of highly efficacious drugs with low adverse side-effects.

1.2.2. Antipsychotic Drugs: A History and Dichotomy of Generations

A. First Generation 'Typical' Antipsychotic Drugs

The early first generation or 'typical' antipsychotic compound, chlorpromazine, was discovered in 1950 by scientists at Rhone-Poulenc Laboratories (Paris, France) (reviewed in Newcomer, 2005; Shen, 1999; Tandon et al., 2010). Chlorpromazine was initially observed to induce prolonged sleep when administered with barbiturates and reduced the required dosage of anaesthetic during medical surgery (Zirkle, 1973). A select group of psychiatrists were soon provided with the drug and reported treatment success in patients exhibiting agitation, and hyperactive, erratic and uncontrollable behaviour (reviewed in Shen, 1999). In 1952, Rhone-Poulenc Laboratories released chlorpromazine as largactil, which was later remarketed as thorazine by Smith, Kline and French (Philadelphia, PA). Thus began the era of first generation antipsychotic drug development and a new hope for the selective treatment of schizophrenia. Chlorpromazine remained a predominant prescription for schizophrenia until the 1970s, however many structurally

similar typical antipsychotics were now available on the market, including haloperidol and fluphenazine (López-Muñoz and Alamo, 2009; Millar, 1963). The discovery of haloperidol by Janssen Pharmaceutica (Turnhout, Belgium) in 1958 also had an immense and long-term impact on the 'psychopharmacological revolution'. It later contributed to the first biologically-based hypothesis on the etiopathology of schizophrenia by providing an experimental model to examine the mechanisms of first generation antipsychotic drug efficacy in the treatment of schizophrenia (reviewed in López-Muñoz and Alamo, 2009).

One of the earliest reports describing an effect of first generation antipsychotics on dopamine neurotransmission dates back to 1963 (Carlsson and Lindqvist, 1963). Research has since identified a correlation between clinical potency and antipsychotic drug binding affinity to the dopamine D2 receptor sub-type, with an occupancy of between 60 – 80% of D2 receptors in the brain inducing a therapeutic response (Kapur, 1998). However, at these levels, D2 receptor antagonism can cause serious motor and extrapyramidal side-effects (EPS) such as tremor, akinesia, slurred speech, tardive dyskinesia and Parkinsonism (Farde et al., 1992). In fact, the link between EPS and an antipsychotic drug's efficacy in treating what was then thought of as schizophrenia in its entirety, was solidified with the introduction of haloperidol and first reports of this correlation date back to 1959 (Hippius, 1989; Shen, 1999). However, it is now generally accepted that first generation antipsychotic drugs have preferential therapeutic effects on the positive symptoms of schizophrenia, which are largely caused by dopaminergic dysregulation in the mesolimbic and mesocortical pathways in the brain (Howes and Kapur, 2009; Tost et al., 2010), but have less therapeutic benefit for the negative and cognitive domains of the disease (Kapur and Remington, 2001). A significant portion of the schizophrenia population is unresponsive to D2 receptor antagonists (Meltzer, 1992), which suggests that D2 blockade alone is insufficient to treat schizophrenia in all cases. Wolkin et al., (1989) found that individuals with

antipsychotic-resistant schizophrenia had the same central uptake of a D2 antagonist ([¹⁸F]N-methylspiroperidol) as antipsychotic-responsive patients following treatment with the typical antipsychotic haloperidol, indicating that the absence of antipsychotic effect in non-responsive schizophrenia was not due to altered D2 receptor binding or drug uptake. Thus, a need was identified for a new line of pharmacological therapeutics that had a broader neurotransmitter receptor binding profile and greater efficacy in treating the positive, negative and cognitive domains of schizophrenia, while addressing the important issue of EPS.

B. Second Generation 'Atypical' Antipsychotic Drugs

In the early 1960s, scientists from Wander Pharmaceuticals (Bern, Switzerland) developed clozapine, an antipsychotic drug with a reduced risk of EPS (Breier et al., 1994; Shen, 1994). Clozapine was effective in treating both the positive and negative symptoms of schizophrenia (Breier et al., 1994) and was efficacious in treating a percentage of individuals that were previously antipsychotic-unresponsive (Kane et al., 1988). However, it was rapidly removed from the market upon reports from Finland that its use was associated with fatal agranulocytosis, an acute and severe lowering of the granulocyte class of white blood cells (Idänpään-Heikkilä et al., 1977). Despite this set-back, excitement over its success in treating previously non-responsive schizophrenia was maintained by a few scientists at Sandoz Laboratories (Switzerland), who had purchased the original developers of the drug, Wander Pharmaceuticals (Shen, 1999). Later, a large-scale, double-blind study by Kane and colleagues (1988) reported clozapine's efficacy and safety in treating non-responsive schizophrenia when individuals were monitored for blood abnormalities, assisting the launch of clozapine onto the USA market in 1990 (Shen, 1999).

Thus began the rapid development of a new 'second generation' of antipsychotic drugs, including olanzapine, clozapine, risperidone, quetiapine and ziprasidone, that were reportedly effective at

treating multiple domains of schizophrenia and showed reduced risk of EPS (Newcomer, 2005). A clear dichotomy had now formed between the older first generation 'typical' and newer second generation 'atypical' antipsychotic drugs. A precise definition of 'atypicality' that allows infallible categorisation of all antipsychotic drugs remains elusive. However, atypical antipsychotic drugs are those that generally possess a profile to affect a range of receptors, notably adrenergic α_1 and α_2 , histaminergic H_1 , muscarinic M_1 and serotonergic $5-HT_{1A}$, $5-HT_{2A}$ and $5-HT_{2C}$, with less preference to dopamine D_2 receptor binding and therefore a lower propensity to cause EPS than first generation drugs (Reynolds, 1998; Richelson and Souder, 2000; Roth et al., 2004) Table 1.1). The recent introduction of aripiprazole adds to the difficulty in differentiating typical from atypical antipsychotics: it acts largely on the dopamine D_2 receptor (similar to first generation typical antipsychotic drugs) but has a low risk of inducing EPS, can treat multiple symptoms of schizophrenia (similar to second generation atypical antipsychotics) and has a low metabolic liability (Naber and Lambert, 2004). Several studies suggest that aripiprazole is a 'functionally selective' antipsychotic drug that may cause different cell signalling cascades through the one receptor sub-type, i.e. an agonist of one pathway and antagonist of another (Mailman and Murthy, 2010; Urban et al., 2006). Aripiprazole may achieve its therapeutic efficacy with reduced side-effects by causing differential actions on the dopamine D_2 receptor (Mailman and Murthy, 2010) in different pathways in the brain (Han et al., 2009). Therefore, aripiprazole may have a different mode of action to other antipsychotic drugs and could be considered a new class of antipsychotic drug – a third generation antipsychotic (Mailman and Murthy, 2010),

The efficacy of second generation antipsychotics to treat the positive, negative and cognitive symptoms of schizophrenia may be related to their broad receptor binding profile (Reynolds, 1998) (Table 1.1). However, drug effects on multiple neural and peripheral pathways may also underlie the numerous adverse side-effects associated with their use (Nasrallah, 2008). Metabolic

Table 1.1: Receptor binding affinities of selected antipsychotic drugs (K_i nM)

Receptor binding affinity (K_i nM) for some common typical and atypical antipsychotics, including first[#], second^{##} and third^{###} generation drugs.

K_i determinations were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2008-00025-C (NIMH PDSP). The NIMH PDSP is Directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA. *Kroeze et al., (2003), **Richelson and Souder (2000), ***Silvestre and Prous (2005)

Receptor	CHLOR [#]	HAL [#]	CLOZ ^{##}	OLAN ^{##}	RISP ^{##}	QUET ^{##}	ZIPR ^{##}	ARI ^{###}
D ₁	112	83	189	58	60	712	30	387
D ₂	2	2	431	72	4.9	567	4	0.95
5-HT _{1A}	3115	1202	105	610 ^{**}	190 ^{**}	431	1.9 ^{**}	5.6
5-HT _{2A}	1.8	61 ^{**}	2.59 ^{**}	1.48 ^{**}	0.15 ^{**}	31 ^{**}	0.12 ^{**}	8.7 [*]
5-HT _{2C}	25	4700 ^{**}	4.8 ^{**}	4.1 ^{**}	32 ^{**}	3500 ^{**}	0.9 ^{**}	22.4 [*]
α ₁	2.6	17 ^{**}	6.8 ^{**}	44 ^{**}	2.7 ^{**}	8.1 ^{**}	2.6 ^{**}	30 ^{***}
α ₂	769.23	600 ^{**}	15 ^{**}	280 ^{**}	8 ^{**}	80 ^{**}	154 ^{**}	70 ^{***}
H ₁	6 [*]	3002	2	4.9	5.18	7.5	130	29
M ₁	47	>10000	14	24	>10000	858	>10000	6776
M ₃	47	>10000	25	51	>10000	1943	>10000	4677
CB ₁	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000

Antipsychotic drug: CHLOR: chlorpromazine, HAL: haloperidol, CLOZ: clozapine, OLAN: olanzapine, RISP: risperidone, QUET: quetiapine, ZIPR: ziprasidone, ARI: aripiprazole.

Receptors: D₁: dopamine₁, D₂: dopamine₂, 5-HT_{1A}: serotonin_{1A}, 5-HT_{2A}: serotonin_{2A}, 5-HT_{2C}: serotonin_{2C}, α₁: adrenergic₁, α₂: adrenergic₂, H₁: histamine₁, M₁: muscarinic₁, M₃: muscarinic₃, CB₁: cannabinoid₁

side-effects such as weight gain, abdominal adiposity, dyslipidaemia, gluco-regulatory abnormalities and insulin-resistance, are commonly observed during SGA treatment (Allison et al., 2009; Newcomer, 2005) and are of growing concern due to associated co-morbidities, such as obesity, type II diabetes mellitus and cardiovascular disease, and the increasing popularity of this drug class (Centorrino et al., 2002; Hollingworth et al., 2010; Vitiello et al., 2009; Zuddas et al., 2011). Unfortunately, people with schizophrenia have a 1.5-2.0 times higher incidence of obesity and type II diabetes mellitus than the general population (American Diabetes Association et al., 2004) and weight gain is an important contributing factor to medication non-compliance (Weiden et al., 2004). These critical issues highlight the importance and urgency of understanding the mechanisms underlying antipsychotic-induced metabolic side-effects.

The SGA olanzapine is commonly used in the clinical setting due to its efficacy to treat the multiple domains of schizophrenia, its tolerability and low patient discontinuation rate (Haro et al., 2009; Lieberman et al., 2005). In addition, wide-spread prescription for bipolar disorder, major depression, dementia, Alzheimer's disease, anorexia nervosa and Tourette's syndrome has been reported in adults and children (Ballard et al., 2006; Brambilla et al., 2007; Bridle et al., 2004; Budman et al., 2001; Centorrino et al., 2002; De Hert et al., 2011; Frenchman, 2005; Zuddas et al., 2011). However, with the exception of clozapine, olanzapine has the highest weight gain and diabetogenic liability of all other antipsychotic drugs (Allison et al., 2009; Newcomer, 2005; Silvestre and Prous, 2005). A study by Zipursky et al., (2005) found that individuals treated with olanzapine at the normal dosage range of 5-20mg/day gained 9.2kg within the first 12 weeks of therapy, increasing to 15.5kg after 52 weeks and plateauing at 15.4kg after 2 years. Another study reported a mean estimated body weight gain of 4.45kg and 4.15kg following 10-weeks clozapine and olanzapine treatment, respectively (Allison et al., 1999). Chronic olanzapine

treatment is also associated with hyperinsulinaemia, hepatic insulin-resistance and type II diabetes mellitus (Oriot et al., 2008; Perez-Iglesias et al., 2008). Even short-term (2-weeks) olanzapine treatment can induce insulin dysregulation in individuals with schizophrenia (Chiu et al., 2010). Some potential mechanisms of SGA-induced metabolic dysfunction have emerged in the past few years, including from our laboratory (Deng et al., 2010; Deng et al., 2007b; Han et al., 2008a; Huang et al., 2006a; Huang et al., 2006b; Weston-Green et al., 2008) and others (see reviews by Coccorello and Moles, 2010; and Reynolds and Kirk, 2010). However, an indisputable hypothesis remains elusive, perhaps because antipsychotic drugs differ markedly in their pharmacological profiles, and because of the complex and well-preserved interactions between the systems involved in energy homeostasis. Olanzapine may interfere in a number of signalling pathways to cause metabolic abnormalities. Therefore, investigation into drug effects on key regulators of energy homeostasis in regions of the brain involved in metabolic control is crucial in order to better understand the mechanisms underlying these metabolic side-effects.

1.2.3 The Control of Energy Homeostasis

I am hungry, therefore I eat. This seemingly simple concept is the outside mask of an elaborate and complex web of well-preserved interactions between multiple neural and peripheral networks that signal through a swarm of hormones, peptides and neurotransmitters to integrate information about energy stores and requirements in order to maintain energy homeostasis. This section of the review will focus on some promising targets that may assist in understanding the mechanisms of antipsychotic drug-induced metabolic dysfunction.

A. Metabolic Hormonal Signals and the Effect of Antipsychotic Drugs

Appetite and satiety signals, such as orexigenic ghrelin and anorexigenic insulin, CCK and PYY, are secreted on a meal-to-meal basis to signal the body's acute energy requirements, whereas

leptin is secreted from adipocytes in direct proportion to fat mass and signals the long-term energy stores of the body to the brain (Orr and Davy, 2005). Metabolic hormones exert their effects primarily on the hypothalamus and caudal brainstem to promote or suppress feeding behaviour. Hormonal imbalances and/or resistance are associated with obesity and diabetes (Schwartz and Porte, 2005; Wren and Bloom, 2007). Antipsychotic drugs may influence these hormonal signals and their pathways in the brain to induce metabolic side-effects.

I. Insulin

Insulin lowers glucose levels broadly through two mechanisms: facilitating glucose uptake and suppressing hepatic glucose production (Bouche et al., 2004). Insulin is secreted from pancreatic β -cells during two phases of digestion: the cephalic phase (i.e. anticipatory) in order to prime the body for glucose absorption, and the gastric phase (i.e. post-absorptive) in response to increasing blood glucose levels (Ahrén, 2000) (discussed further on page 23). Insulin facilitates glucose uptake into gluco-responsive tissues, such as the liver and skeletal muscle, and maintains blood glucose homeostasis. It also acts on various regions of the brain, particularly the hypothalamus and brainstem, which are abundant in insulin receptors (Pardini et al., 2006; Unger et al., 1991). Insulin can inhibit food intake, in part, by suppressing expression of the potent orexigen, neuropeptide Y (NPY) (Schwartz et al., 1992). It is commonly classified as an adiposity signal, along-side leptin, as insulin levels in the absence of glucose stimulation are proportional to adiposity (Baskin et al., 1999; Polonsky et al., 1988; Schwartz et al., 2000).

Studies have shown that insulin is excessively elevated following food intake in human obesity and remains elevated post-prandially past 2 hours, unlike lean subjects whose insulin levels return to basal readings after 2 hours (Polonsky et al., 1988; Zwirska-Korczala et al., 2007). Chronically elevated insulin levels and concurrent hyperglycaemia may be indicative of insulin-

resistance, and are characteristic symptoms of type II diabetes mellitus (Ahrén, 2000). In addition, the link between obesity and insulin-resistance is well-documented (Belfiore and Iannello, 1998; Goossens, 2008), although insulin-resistance and diabetes can also occur independent of excessive body weight (Arner et al., 1991).

Chronic olanzapine treatment increases plasma insulin and induces insulin-resistance in humans (Oriot et al., 2008; Perez-Iglesias et al., 2008; Sacher et al., 2008; Wu et al., 2008), rats (Albaugh et al., 2006), and mice (Coccurello et al., 2009), while restoration of normal glycaemic control has been reported following discontinuation of antipsychotic administration (Koller et al., 2001). Even a single acute dose of olanzapine, risperidone or clozapine can cause hyperglycaemia and hyperinsulinaemia, impair insulin-sensitivity, and induce insulin-resistance (Boyda et al., 2010b; Houseknecht et al., 2006). Reports also show that olanzapine treatment can decrease glucose-stimulated insulin levels in the clinic following short-term (14-days) olanzapine treatment (Chiu et al., 2006), and in male rats following a single acute dose of olanzapine (Chintoh et al., 2008a). Olanzapine and clozapine, both with a high diabetogenic liability, can directly decrease insulin secretion from pancreatic β cells *in-vitro*, whereas ziprasidone and risperidone, SGAs with a lower risk of inducing type II diabetes mellitus, have no effect on insulin secretion (Johnson et al., 2005). The ability of clozapine and olanzapine to decrease insulin secretion in the short-term may be related to their receptor binding profile, for example both drugs are potent antagonists of the cholinergic muscarinic M3 receptor (M3R) (Bymaster et al., 1999; Johnson et al., 2005), which facilitates insulin production and secretion from pancreatic β cells (Johnson et al., 2005; Ruiz de Azua et al., 2011). Therefore, direct blockade of the M3R by olanzapine may interfere in insulin levels in the body. Further review of the M3R and its potential role in olanzapine-induced insulin dysregulation is detailed on pages 26 and 27.

II. Ghrelin

Ghrelin is a hunger hormone primarily released from endocrine cells in the stomach to stimulate feeding. In addition to the stomach, ghrelin has been detected in a number of other tissues such as the pancreas and brain, albeit at lower levels (Hou et al., 2006; van der Lely et al., 2004). Ghrelin exerts its orexigenic effects through the growth hormone secretagogue receptor (GHS-R) (Cowley et al., 2003; Kojima et al., 1999; Kojima and Kangawa, 2005). It enters the brain and acts on regions such as the hypothalamus and brainstem to promote food intake (Cone et al., 2001). Circulating ghrelin peaks immediately prior to a meal, then decreases rapidly post-prandially (Tschop et al., 2001). Peripheral ghrelin administration induces food intake in humans (Wren et al., 2001), and central and peripheral administration promotes food intake, weight gain and adiposity in rats (Wren et al., 2000). Fasting plasma ghrelin concentrations are reduced in human obesity compared to lean controls (Shiia et al., 2002; Tschop et al., 2001; Zwirska-Korczala et al., 2007), indicating a possible compensatory down-regulation of the orexigenic hormone during a chronic positive energy state.

The reported effects of atypical antipsychotic drugs on circulating ghrelin levels are inconsistent (Atmaca et al., 2007; Esen-Danaci et al., 2008; Kluge et al., 2009; Tanaka et al., 2008), (also see Jin et al., 2008 for review). For example, studies have shown that treatment with olanzapine, clozapine and risperidone significantly elevates fasting bioactive ghrelin (n-octanoylated ghrelin) levels in humans and rats (Esen-Danaci et al., 2008; Murashita et al., 2007; Murashita et al., 2005). The finding that ghrelin is increased following SGA treatment coincides neatly with the elevated risk of weight gain side-effects of these drugs and suggests dysfunction of central feedback mechanisms or over-secretion of the hormone that induces hyperphagia. Jin and colleagues (2008) compiled the results of 8 studies on ghrelin following SGA treatment in humans and found that 3 studies reported an increase in ghrelin, 2 reported a decrease, while 3 studies

showed no change in circulating ghrelin levels. There is a need to investigate the effects of SGAs on ghrelin using an appropriate animal model that eliminates some of the variables associated with human studies, such as drug dosage, treatment duration, concomitant treatments, starting BMI, age and family history (Gebhardt et al., 2009).

III. Cholecystokinin (CCK)

CCK is an anorectic hormone that is predominantly secreted by endocrine I cells in the mucosa of the duodenum, jejunum and proximal ileum of the upper intestine in response to amino and fatty acid absorption through the intestinal mucosal wall (Buchan et al., 1978). It signals the body's satiated state to the brain by binding to receptor sub-types 1 and 2 (CCK1R and CCK2R, formerly CCK-A and CCK-B) on vagal nerve afferents that project to the brainstem (Dockray, 2009), and through CCK-2R receptors in the hypothalamus (Orr and Davy, 2005). CCK can also influence food intake by directly acting on the myenteric plexus (Cooper et al., 2008b). CCK signalling is altered in clinical obesity and in obese animal models, i.e. fasting and postprandial CCK levels decreased in morbidly obese women compared to lean controls (Zwirska-Korczala et al., 2007), and were reduced in the hypothalamus of mice fed a high-fat diet for 10 weeks (Morris et al., 2007). In addition, peripheral administration of CCK decreases meal size and frequency in humans, rats and mice (Geary, 2004; Gutzwiller et al., 2000; Lieveise et al., 1995), and plays an important role in stimulating pancreatic secretion (Konturek et al., 2003)

Reports on the effects of antipsychotics on CCK levels are limited. Vidarsdottir et al. (2010) found a slight pre-prandial morning increase in plasma CCK levels in healthy male olanzapine-treated subjects, and an association between polymorphisms in the CCK2R receptor and antipsychotic-induced weight gain has been suggested (Tiwari et al., 2010a). However, the effects of antipsychotic drugs on circulating CCK in an animal model of metabolic dysfunction are unknown.

IV. Peptide Tyrosine Tyrosine (PYY)

PYY is referred to as the 'ileal brake' on food intake and is released by endocrine cells in the ileum in response to fatty acids present in the intestine (Chaudhri et al., 2005). PYY₍₃₋₃₆₎, an endogenous form of PYY, has a high affinity for appetite-influencing NPY Y1 and Y5 receptors, and binds irreversibly to Y2 receptors (Batterham and Bloom, 2003; Dautzenberg and Neysari, 2005). The hypothalamus and brainstem express NPY receptors and may play a role in mediating the anorexigenic effects of PYY (Fetissov et al., 2004). In particular, the caudal brainstem has a high density of Y2 receptors in the brain (Dumont et al., 1996). Fasting and postprandial plasma PYY levels are reduced in human obesity (Batterham et al., 2003; Zwirski-Korczala et al., 2007) and peripheral PYY administration can attenuate food intake by approximately 30% in both lean and obese humans (Batterham et al., 2003). Taken together, these findings illustrate a role for PYY in the pathogenesis of obesity, and highlight its potential candidature for obesity therapeutics, as humans do not appear to be resistant to its satiating effects (Batterham et al., 2003).

Olanzapine treatment alters PYY binding in regions in the brain, including the medial amygdaloid nucleus, medial geniculate nucleus and superficial gray layer of the superior colliculus, parabrachial pigmented nucleus and periaqueductal gray (Wang and Huang, 2008). Vidarsdottir et al. (2010) reported no change in circulating PYY levels in healthy olanzapine-treated male subjects. However, the effects of olanzapine on PYY in an animal model of antipsychotic-induced metabolic dysfunction have not been reported.

V. Leptin

Leptin is predominantly expressed in adipocytes by the *ob* gene and circulating leptin levels parallel adiposity. Leptin enters the brain to act on regions such as the hypothalamus and brainstem to reduce food intake (Vong et al., 2011), though a reduction in leptin can signal depleted energy storage and induce hyperphagia (Zhang et al., 1994). Exogenous application of leptin results in hypophagia and weight loss, as well as increased energy expenditure in mice (Zhang et al., 1994). Mutated leptin signalling, i.e. obese (*ob/ob*) mice that lack a functional leptin gene and diabetic (*db/db*) mice that lack leptin receptors, results in an obese phenotype, while the application of recombinant leptin to leptin-mutant strains induces weight loss (Geary, 2004; Halaas et al., 1995; Pelleymounter et al., 1995), identifying leptin as an important regulator of body weight. These finding that leptin could reduce body weight initially sparked excitement for the possible therapeutic benefits of leptin treatment for human obesity (Friedman and Halaas, 1998). However, excitement was soon quelled by the observation that morbidly obese human subjects had high plasma leptin concentrations, despite high body mass index (BMI), and some were unresponsive to exogenous leptin administration (Heymsfield et al., 1999). It is now generally accepted that obesity is a condition of leptin-resistance rather than deficiency, due to the failure of leptin to activate key satiety signalling pathways and attenuate body weight gain in clinical obesity, despite chronically high levels of the hormone (Caro et al., 1996; Heymsfield et al., 1999; Maffei et al., 1995; Morrison, 2008).

As with ghrelin, studies on the effects of atypical antipsychotics on fasting plasma leptin levels appear to be conflicting. For example, Jin and colleagues (2008) reviewed 30 papers dated between 1998 and 2007 and found that most studies agreed on a SGA-induced increase in circulating leptin, which commonly correlated to BMI or body fat mass. However, 11 papers revealed no change in leptin, particularly when data was adjusted for BMI, while 1 showed a

decrease (Jin et al., 2008). Confounding factors such as body mass index at the time of testing, antipsychotic dosage, length of treatment, concomitant treatments, treatment history and history of illness may contribute to this contrast in results (Gebhardt et al., 2009).

As leptin is secreted by adipocytes to signal the quantity of fat stores, it has been proposed that hyperleptinaemia during antipsychotic treatment may be reflective of weight gain/adiposity and not a direct drug action (Jin et al., 2008). However, several studies indicate that olanzapine may directly increase fat reserves, for example, olanzapine induces adipogenesis *in-vitro* through enhanced expression of sterol regulatory element-binding protein 1, which together with its related genes promotes the production of adipocytes (Yang et al., 2007). Olanzapine also enhances differentiation of pre-adipocytes to mature adipocytes and triglyceride accumulation *in-vitro* (Yang et al., 2007). In male rats, olanzapine enhances fuel uptake in adipocytes and attenuates lipolysis (Albaugh et al., 2010).

B. The Brain-Gut Axis

If you've ever felt a sudden pang of hunger after looking at an appetising meal, you know that the stomach listens attentively to the brain. Likewise, when you are full, food seems less appetising as the brain begins to listen to the stomach. The digestive system closely interacts with the brain in a well-preserved bi-directional manner to convey multitudes of chemical and electrical messages pertaining to energy stores, nutritional value of incoming food, mechanical distension, hunger and satiety. This system is referred to as the brain-gut axis (Figure 1.1). The hypothalamic arcuate nucleus (Arc) and the dorsal vagal complex (DVC) of the caudal brainstem form important components of the brain-gut axis by integrating information from peripheral metabolic hormonal signals originating from the gastrointestinal (GI) tract and adipocytes, as well as

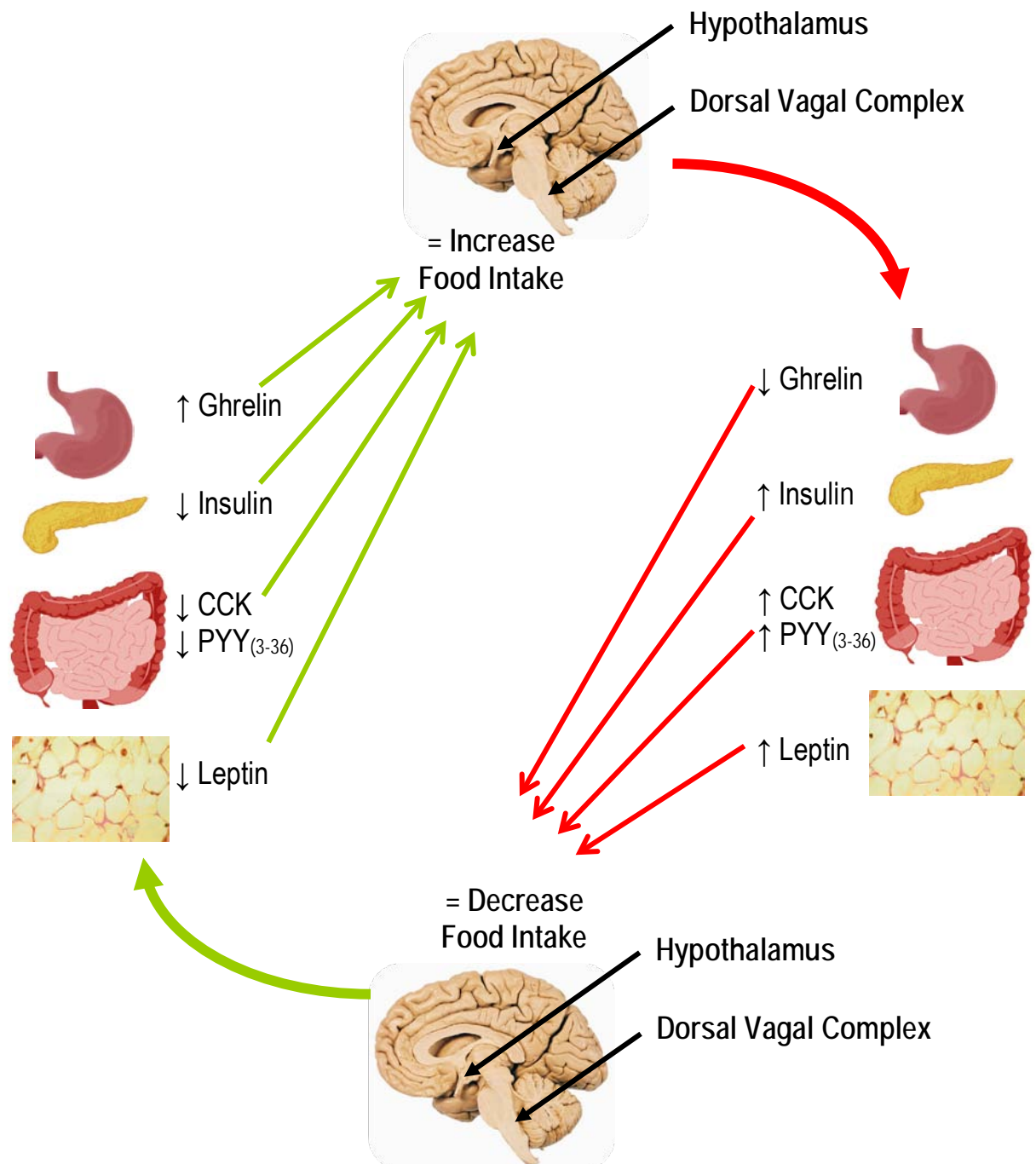


Figure 1.1: Schematic of the Brain-Gut Axis.

Food intake decreases orexigenic ghrelin from the stomach and increases anorexigenic hormones such as cholecystokinin (CCK) and peptide YY₍₃₋₃₆₎ (PYY₍₃₋₃₆₎) from the upper intestine. Glucose is absorbed into the blood stream and stimulates pancreatic release of insulin, which acts to restore normal glycaemic levels by promoting glucose uptake and inhibiting food intake. During periods of high energy intake leptin is released from adipocytes to signal enhanced fat mass. These signals converge on neurons of the hypothalamus and the dorsal vagal complex of the caudal brainstem to convey information pertaining to the body's acute and long-term nutritional status. During periods of low energy the brain stimulates feeding behaviour that is associated with an increase in ghrelin and reduced levels of insulin, CCK, PYY₍₃₋₃₆₎ and leptin. This feedback mechanism acts to maintain energy homeostasis, and dysfunction of the brain-gut axis can result in a shift in energy balance associated with obesity or anorectic eating disorders.

neurotransmitter signalling from other brain regions, resulting in the overall stimulation or inhibition of food intake (Berthoud, 2002; Orr and Davy, 2005). Peripheral hormones can signal to the brain by either passing through the blood-brain barrier to their respective neuronal receptors, or by acting on receptors expressed on vagal afferent fibres from the GI tract to the brain (reviewed in Dockray, 2009). Thus, the hypothalamus and brainstem represent parallel systems for integrating neural and peripheral information crucial for maintaining energy homeostasis, and manipulation of metabolic signals and their receptors in these regions may be prime targets for the prevention or treatment of antipsychotic-induced metabolic dysfunction.

C. The Neural Control of Appetite and Glucose Homeostasis

The neural control of appetite involves integration of multiple pathways in the brain including those pertaining to the metabolic, emotional, cognitive, memory, sensory and anticipatory/reward motivators of feeding behaviour. Thus, the desire to eat, or lack thereof, engages most regions of the brain, from the brainstem through to the hippocampus and cortex (Shin et al., 2009; Stice et al., 2009). The hypothalamus and the caudal brainstem are well-recognised as key regions facilitating energy homeostasis, integrating and responding to metabolic hormone signals based on the body's acute and long-term energy stores and requirements (Berthoud, 2002; Broberger and Hökfelt, 2001).

I. The Role of the Hypothalamus and Caudal Brainstem in Energy Homeostasis

Classic studies identified the ventromedial hypothalamic (VMH) nucleus as the 'satiety centre', while the lateral hypothalamic area (LHA) was deemed the 'hunger centre' (reviewed in Bray et al., 1990; Stellar, 1954), however the theory of a single appetite centre has since been expanded (Shin et al., 2009). The hypothalamic Arc is an important modulator of energy homeostasis and may influence both sides of the energy equation: food intake and physical activity (Coppari et al.,

2005). The Arc is a circumventricular organ that is situated adjacent to the third ventricle and projects through the overlying permeable medial eminence, allowing circulatory sampling via access to the portal vascular system and cerebrospinal fluid, in order to integrate and respond to peripheral metabolic hormonal signals (Cone et al., 2001; Peruzzo et al., 2000). The Arc also receives information from an abundance of intrahypothalamic connections, including from the lamina terminalis and the subfornical organ located in the anterior hypothalamus, which carry information about olfactory, visual, taste and reward aspects of feeding (Berthoud, 2002; Wuchert et al., 2009). In addition, the Arc also projects to other brain regions important in the regulation of energy homeostasis, including the VMH (Chee et al., 2010), and the dorsal vagal complex (DVC) of the caudal brainstem (Berthoud, 2008; Broberger and Hökfelt, 2001; Gray et al., 1986; Hou et al., 2006).

The DVC, comprised of the dorsal motor nucleus (DMN), nucleus of the solitary tract (NTS) and the area postrema (AP), can respond to circulating metabolic hormones through the permeable AP that borders the fourth ventricle (Wuchert et al., 2009). In addition, projections between the Arc, DVC and the vagus nerve allow direct regulation of gastrointestinal function and feeding (Berthoud, 2008; Gray et al., 1986; Hou et al., 2006; Tebbe et al., 2001; Travagli et al., 2006; Zheng et al., 2005). Furthermore, vagal afferents from the alimentary canal provide rapid information to the brainstem and hypothalamus pertaining to the nutritional value of food in the GI tract, circulating and stored fuel status, mechanical GI signals such as gastric distension, as well as the regulation of circulating metabolic hormones including insulin, ghrelin and CCK (Berthoud, 2008; Gray et al., 1986; Tebbe et al., 2001). The DVC receives gastrointestinal afferent fibres that travel to the brain via the vagus nerve, medulla and solitary tract to terminate on neuronal cell bodies and dendrites of the NTS (Rogers et al., 1996; Travagli et al., 2006). NTS inter-neurons then project to the efferent vagal preganglionic neurons of the DMN, which descend and synapse

on intra-ganglionic fibres of the abdominal viscera (Rogers et al., 1996; Travagli et al., 2006). Signalling between NTS afferents and DMN efferents is dynamic and sensitive to influences such as hormones, neurotransmitters and synaptic signalling from other brain regions, including the hypothalamus (Gray et al., 1986; Hou et al., 2006; Travagli et al., 2006). Altered efferent output can have potent effects on the modulation of gastrointestinal function and energy homeostasis (Gray et al., 1986; Hou et al., 2006; Travagli et al., 2006), for example, high densities of post-ganglionic fibres originating from the DMN terminate in the stomach and alterations in DMN signalling can influence gastric motility, which effects glycaemia and insulin levels by altering the absorption rate of glucose (reviewed in Berthoud et al., 1991; Gilon and Henquin, 2001; Okumura and Namiki, 1990; Travagli et al., 2006).

Pro-opiomelanocortin (POMC) and Neuropeptide Y (NPY)

The hypothalamic Arc contains two distinct 'first-order' neuronal populations: a medially-positioned group of orexigenic neurons co-expressing NPY and agouti-related protein (AgRP), and an opposing anorexigenic cluster co-expressing pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) localised in the lateral part of the Arc. POMC and NPY mRNA are also expressed in the DVC (Bronstein et al., 1992; Wai et al., 2004), and NPY mRNA expression has been reported in the VMH (Huang et al., 2006b). The POMC gene encodes for neuropeptides such as β -endorphin, adrenocorticotrophic hormone and α -melanocortin stimulating hormone (α -MSH); the latter exerts its anorexigenic effects largely through melanocortin MC-3 and MC-4 receptor (MC4-R) subtypes. Conversely, the central application of NPY induces food intake in a number of species and chronic over-exposure to NPY leads to obesity in rats (Beck et al., 1992; Stanley et al., 1986), while NPY inhibition can attenuate feeding and body weight (reviewed in Minor et al., 2009). NPY exerts its effects through G-protein coupled NPY receptors, termed Y₁₋₅, located throughout the brain including the hypothalamus

and brainstem (Dumont et al., 1996; Fetissov et al., 2004). NPY $Y_{1,2,4}$ and Y_5 receptors are implicated in the regulation of food intake (Abbott et al., 2005; Kalra and Kalra, 2003; Yahya et al., 2006). Genetic animal models of hyperphagia and obesity, such as Zucker fatty rats (*fa/fa*), obese (*ob/ob*) and diabetic (*db/db*) mice, exhibit enhanced hypothalamic NPY and reduced POMC mRNA expression (Beck et al., 1993; Kim et al., 2000; reviewed in Minor et al., 2009). NPY/AgRP neurons release gamma-aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the central nervous system, from axonal terminals that synapse on POMC neurons in order to stimulate feeding behaviour (Cone et al., 2001). In the Arc, POMC neurons are also inhibited by local non-NPY/AgRP GABAergic neurons but are disinhibited by the application of leptin, which acts directly on these newly characterized presynaptic GABAergic neurons to suppress inhibitory tone to POMC (Vong et al., 2011). Indeed, the appetite enhancing effects of hypothalamic GABA were reported more than 30 years ago (Meeker and Myers, 1980), however, to-date, there has been minimal research into the underlying mechanisms (Wu and Palmiter, 2011). NPY and POMC neurons are sensitive targets for a number of peripheral metabolic hormones including leptin, insulin, ghrelin, CCK and PYY (Cone et al., 2001; Meister, 2007; Valassi et al., 2008; van den Top et al., 2004), allowing response to the acute nutritional status of the body and the long-term regulation of energy stores. For example, activation of ghrelin receptors expressed on NPY neurons stimulates inhibitory GABA release onto POMC neurons to promote appetite (Cone et al., 2001), whereas leptin depolarizes and increases the firing rate of POMC neurons while reducing NPY-mediated GABAergic inhibition of POMC (Cowley et al., 2001; van den Top et al., 2004). NPY also has a robust effect on locomotor activity as studies have shown that intracerebroventricular administration of NPY in rats significantly attenuates home cage activity and open-field locomotion in a reversible manner (Heilig and Murison, 1987; Heilig et al., 1988). Other authors have also reported altered locomotor activity following intervention into NPY signalling by pharmacological and genetic manipulation (Costoli et

al., 2005; Edelsbrunner et al., 2009a; Edelsbrunner et al., 2009b; Heilig et al., 1989; Karl et al., 2008; Maric et al., 2009; Pedrazzini et al., 1998). In particular, the Arc, which houses a large quantity of NPY neurons, has been identified as target for leptin-mediated locomotor regulation (Coppari et al., 2005). Taken together, the literature has revealed an important role for the Arc and DVC as a target site for numerous satiety and hunger signals, and an integral modulator of appetite. NPY and POMC neurons can regulate energy homeostasis through opposing influences on appetite and, in-part, physical activity. Therefore, antipsychotic drug effects on NPY or POMC expression may cause a significant shift in energy balance that favours body weight gain. POMC genetic variations have been described as a susceptibility for antipsychotic-induced body weight gain (Correll and Malhotra, 2004). However, investigation into the effects of antipsychotic drugs on NPY or POMC signalling in the hypothalamus and brainstem is limited.

II. The Central Control of Insulin Secretion and Glucose Homeostasis

The maintenance of glucose levels tightly within the normal range of 4.5 and 6mM involves a fine balance of physiological, cellular and molecular alterations (Ahrén, 2000; Gilon and Henquin, 2001). A number of reviews have highlighted the role of the brain in glucose homeostasis and insulin secretion (Bereiter et al., 1981; Burcelin, 2010; Kalra, 2008; Konturek et al., 2003; Schwartz and Porte, 2005; Thorens, 2010; Woods et al., 1985). Indeed, the influence of the CNS on insulin secretion was recognised by Pavlov as early as 1910 (Pavlov, 1941). Other classical studies identified glucose-stimulated hyperinsulinaemia following VMH lesioning (Frohman et al., 1969; Hales and Kennedy, 1964; Rohner-Jeanrenaud and Jeanrenaud, 1980) that was reversed immediately following bilateral sub-diaphragmatic vagotomy (Bereiter et al., 1981; Berthoud and Jeanrenaud, 1979). Collectively, these studies indicated a regulatory role for the hypothalamus in insulin secretion via the vagus nerve. More recently, early neuronal activity (via *c-fos* expression) has been detected in the Arc, VMH and paraventricular nucleus (PVN) following an acute

intracarotid injection of glucose at a concentration insufficient to increase peripheral glucose levels (Guillod-Maximin et al., 2004), and *c-fos* expression was detected in neurons of the DMN and NTS of the DVC following insulin-induced hypoglycaemia (Yuan and Yang, 2002). These findings show that specific brain regions are activated in response to glucose levels, in particular, select nuclei of the hypothalamus and brainstem. In fact, emerging theory suggests that diabetes is a disease of the central nervous system as alterations in hypothalamic and brainstem signalling promote abnormal glucose metabolism and insulin function characteristic of insulin-resistance and type II diabetes mellitus (Elmqvist and Marcus, 2003; Obici et al., 2003; Obici et al., 2002; Schwartz, 2001). Therefore, these regions are of interest in the pathogenesis of antipsychotic-induced insulin dysfunction and type II diabetes mellitus side-effects.

The Cephalic and Gastric Phases of Insulin Secretion

Insulin is secreted in response to two phases of digestion: the cephalic phase, which occurs prior to nutrient absorption based on sensory input to the brain in anticipation of feeding, and the gastric phase that occurs in response to nutrient absorption from food present in the GI tract. The cephalic phase of insulin secretion is largely reliant on sensory (i.e. visual and olfactory) and oropharyngeal innervation via cranial nerves derived from the brainstem (Ahrén, 2000; Gilon and Henquin, 2001). It acts to prime the body for glucose absorption by preparing the liver, muscles and adipose tissue for glucose uptake. It may also function to reduce the post-prandial load on pancreatic β -cells (Gilon and Henquin, 2001). Diabetes is associated with impaired cephalic phase response, which contributes to hyperglycaemia due to improper nutrient detection (Burcelin, 2010; Knauf et al., 2008). The cephalic insulin response is partially regulated by vagal cholinergic mechanisms, as response is suppressed by vagotomy and the application of atropine, a muscarinic receptor antagonist (Gilon and Henquin, 2001). Conversely, the gastric phase of insulin secretion occurs when absorbed glucose present in the hepatic portal vein activates

enteric glucose sensors that enhance vagal firing to the brain (Gilon and Henquin, 2001; Knauf et al., 2008; Thorens, 2010). The NTS, Arc and VMH contain early response neurons to intragastric glucose infusion (detected via *c-Fos* expression) (Knauf et al., 2008), and efferent pathways derived from the hypothalamus and brainstem mediate an increase in vagal efferent nerve firing to the pancreas during insulin-induced hypoglycaemia (Wu et al., 2004). Taken together, these studies indicate an important role for the hypothalamus and brainstem in both the cephalic and absorptive phases of insulin secretion that involves cholinergic vagal innervation of the pancreas.

III. Neurotransmitter Signalling Pathways in Metabolism

As discussed above, the DVC and hypothalamus play important roles in regulating energy homeostasis, therefore, neurotransmission within these regions is of interest in the pathophysiology underlying antipsychotic-induced metabolic side-effects. A number of neurotransmitter systems are associated with food intake and body weight regulation, including noradrenergic, serotonergic, dopaminergic and histaminergic signalling pathways (Meister, 2007). The endogenous cannabinoid system is also well-documented concerning its regulatory role in energy homeostasis (Bermudez-Silva et al., 2010; Li et al., 2011; O'Hare et al., 2011) and we previously reported that olanzapine induced a robust decrease in cannabinoid receptor binding density in the DVC that correlated to body weight gain (Weston-Green et al., 2008). Furthermore, the muscarinic signalling pathway can regulate metabolic hormones through cholinergic-mediated vagal innervation of the gastrointestinal tract (Lee et al., 2002; Liddle, 2000) and is a key modulator of pancreatic insulin production and secretion (Ruiz de Azua et al., 2011). Therefore, the cannabinoid and muscarinic signalling pathways are prime candidates in the pathogenesis of antipsychotic-induced obesity and diabetes side-effects.

a. Cholinergic Muscarinic Neurotransmission: Role in Glucose and Energy Homeostasis, and Antipsychotic-Induced Metabolic Dysfunction

Acetylcholine (ACh) is an important neurotransmitter of the central and peripheral nervous system and exerts its effects through ionotropic nicotinic and metabotropic muscarinic receptors. Plasma membrane-bound muscarinic ACh receptors are widely expressed throughout the brain and periphery, with dominant expression in the parasympathetic nervous system. Five muscarinic receptor genes have been classified to-date, denoted M₁-M₅ (Caulfield and Birdsall, 1998). Muscarinic receptors have a number of roles in the gastrointestinal tract, including secretory, i.e. insulin and gastric acid secretion, and smooth muscle contraction, for example regulation of gastric motility and peristalsis (Ochi et al., 2005; Ruiz de Azua et al., 2011; Travagli et al., 2006). ACh has been identified in the hypothalamus and brainstem, for example more than 95% of DMN neurons are immunoreactive for choline acetyl-transferase, the ACh-synthesising enzyme (Armstrong et al., 1990; Travagli et al., 2006). ACh is also present in the Arc and overlying median eminence, and choline acetyl-transferase and vesicular ACh transporter immunoreactivity has been reported in POMC neurons of the Arc (Meister et al., 2006; Walaas and Fonnum, 1978). Given the role of the hypothalamus and brainstem in appetite and gastrointestinal function via the vagus nerve, cholinergic neurotransmission in these regions may be implicated in the metabolic abnormalities observed during SGA treatment through drug interactions with muscarinic receptors.

i. Parasympathetic and Sympathetic Regulation of Insulin Secretion

Parasympathetic and sympathetic regulation of pancreatic secretion plays a major role in glucose homeostasis. Sympathetic innervation inhibits insulin secretion from pancreatic islet β -cells during periods of hypoglycaemia and stimulates α -cells to release glucagon (partly mediated by ACh) (Ahrén, 2000). Glucagon promotes the conversion of stored glycogen in the liver to glucose, which is released into the circulation. Conversely, parasympathetic vagal activation stimulates

insulin secretion from β -cells in the pancreas. Parasympathetic pre-ganglionic fibres arise largely in the DMN of the DVC, descend via the hepatic and gastric branches of the vagus nerve to enter the pancreas along the pancreatic vessels and terminate at the intra-pancreatic ganglia dispersed throughout the exocrine pancreas (Ahrén, 2000; Buijs et al., 2001). Post-ganglionic nerve fibres arising from the intrapancreatic ganglia penetrate the pancreatic islets and terminate proximal to the endocrine cells (Ahrén, 2000; Gilon and Henquin, 2001). Vagal nerve stimulation causes the preganglionic nerve terminal to release ACh, which activates nicotinic receptors expressed on the intraganglionic neuron (Ahrén, 2000; Gilon and Henquin, 2001). Activation of the post-ganglionic fibres causes the release of several neurotransmitters, including ACh, pituitary adenylate cyclase activating polypeptide, vasoactive intestinal polypeptide and gastrin-releasing peptide, that bind to their respective receptors expressed on β -cells to influence insulin production and secretion (Ahrén, 2000; Ruiz de Azua et al., 2011).

ii. The Muscarinic M3 Receptor

The M3R is a G protein-coupled receptor (GPCR) belonging to the $G_{q/11}$ -coupled receptor sub-family (Ahren, 2009). It is abundantly expressed by pancreatic β -cells and activates the glucose-stimulated ACh pathway for insulin secretion through two second messenger cascade systems, diacylglycerol and inositol 1,4,5-trisphosphate (Ahrén, 2000). The M3R pathway for ACh-mediated insulin secretion in the β -cell is reviewed in Ruiz de Azua et al. (2011). The physiological importance of the cholinergic M3R in energy balance is evidenced in genetic mutational studies where M3R-deficient mice exhibit a lean phenotype, are hypophagic, hypoglycaemic, hypoinsulinaemic, and have reduced glucagon levels (Duttaroy et al., 2004; Yamada et al., 2001; Zawalich et al., 2004). Although glucose metabolism is unaltered in hepatocyte-specific M3R-deficient mice (Li et al., 2009), mice selectively deficient in M3R expressed on pancreatic β -cells exhibit decreased serum insulin levels and impaired glucose

tolerance compared to their wide-type littermates (Gautam et al., 2006b). In stark contrast, transgenic mice that selectively over-express M3Rs specifically on pancreatic β -cells exhibit a 3 fold increase in insulin secretion accompanied by a 35% decrease in blood glucose levels during fasted and fed states compared to wildtypes (Gautam et al., 2010). In addition, M3Rs are widely expressed throughout the brain, including the VMH, Arc and DVC (Levey et al., 1994; Zubieta and Frey, 1993). Several studies have shown a dynamic sensitivity of central M3Rs to the glucometabolic state of the body, for example increased M3R density has been reported in the brainstem of hyperglycaemic streptozotocin-induced diabetic rats (Balakrishnan et al., 2009), M3R expression is increased in the brainstem of hyperinsulinaemic normoglycaemic rats following a partial pancreatectomy (Renuka et al., 2004), and changes in M3R binding sensitivity in response to glycaemia have been reported in the cerebellum (Antony et al., 2010). Taken together, these findings demonstrate a critical role for cholinergic M3Rs in maintaining glycaemic balance and highlight central M3Rs as promising targets in the mechanisms of SGA-induced type II diabetes mellitus and obesity.

iii. M3 Receptor Role in Antipsychotic-Induced Metabolic Dysfunction

Accumulating evidence over the past decade has shown a role for the muscarinic M1, M2 and M4 receptor sub-types in the pathology of schizophrenia (Dean et al., 2004; Deng and Huang, 2005; Raedler et al., 2007; Scarr et al., 2009) and antipsychotic drug efficacy (Deng et al., 2007b; Scarr and Dean, 2009). However, the M3R does not appear to be involved in the pathology of schizophrenia (Scarr et al., 2006) or the therapeutic effects of antipsychotics (Bymaster and Felder, 2002). Instead, antipsychotic binding affinity to the M3R is thought to be related to its metabolic side-effects (Coccurello and Moles, 2010; Jindal and Keshavan, 2006; Johnson et al., 2005; O'Neill, 2005; Reynolds and Kirk, 2010; Starrenburg and Bogers, 2009). Indeed, a number of factors may contribute to antipsychotic-induced diabetes and obesity side-effects (reviewed in

Coccurello and Moles, 2010; Reynolds and Kirk, 2010; Starrenburg and Bogers, 2009). However, M3R binding affinity has been identified as a predictor of antipsychotic diabetogenic liability (Silvestre and Prous, 2005) and relates, in part, to weight gain risk (Matsui-Sakata et al., 2005). Interestingly, olanzapine and clozapine, antipsychotic drugs with high diabetogenic and obesogenic liabilities, have a receptor binding profile to potently block the M3R, while low-risk antipsychotics, such as risperidone and ziprasidone, have little effect on the M3R (Johnson et al., 2005) (Table 1.1). Coinciding with their relative risk for diabetes side-effects, olanzapine and clozapine decrease glucose and carbachol (a muscarinic receptor agonist) -stimulated insulin from pancreatic β -cells, whereas risperidone and ziprasidone have no effect on insulin secretion *in-vitro* (Johnson et al., 2005). This finding suggests that olanzapine and clozapine can impair the cholinergic pathway for insulin secretion by blocking the M3R (Figure 1.2).

In the clinic, chronic olanzapine treatment is associated with hyperinsulinaemia, hepatic insulin resistance and type II diabetes mellitus (Oriot et al., 2008; Perez-Iglesias et al., 2008), which can also be modelled pre-clinically (Albaugh et al., 2006; Chintoh et al., 2008b; Coccurello et al., 2009). However, a growing body of evidence suggests that short-term/acute olanzapine and clozapine treatment decreases fasting plasma insulin levels and attenuates glucose-stimulated insulin response (Chintoh et al., 2008a; Chiu et al., 2010; Chiu et al., 2006; Johnson et al., 2005; Oriot et al., 2008). Importantly, a recent report by Chiu et al (2010) noted a time-dependent change in glucose-stimulated insulin response in individuals with schizophrenia, whereby insulin levels decreased during the first two weeks of olanzapine treatment compared to their base-line levels, returned to base-line levels after 4-weeks treatment, and increased following 8-weeks olanzapine treatment. It is possible that direct blockade of the M3R during the initial olanzapine treatment period (2-weeks) causes a decrease in glucose-stimulated insulin secretion, which

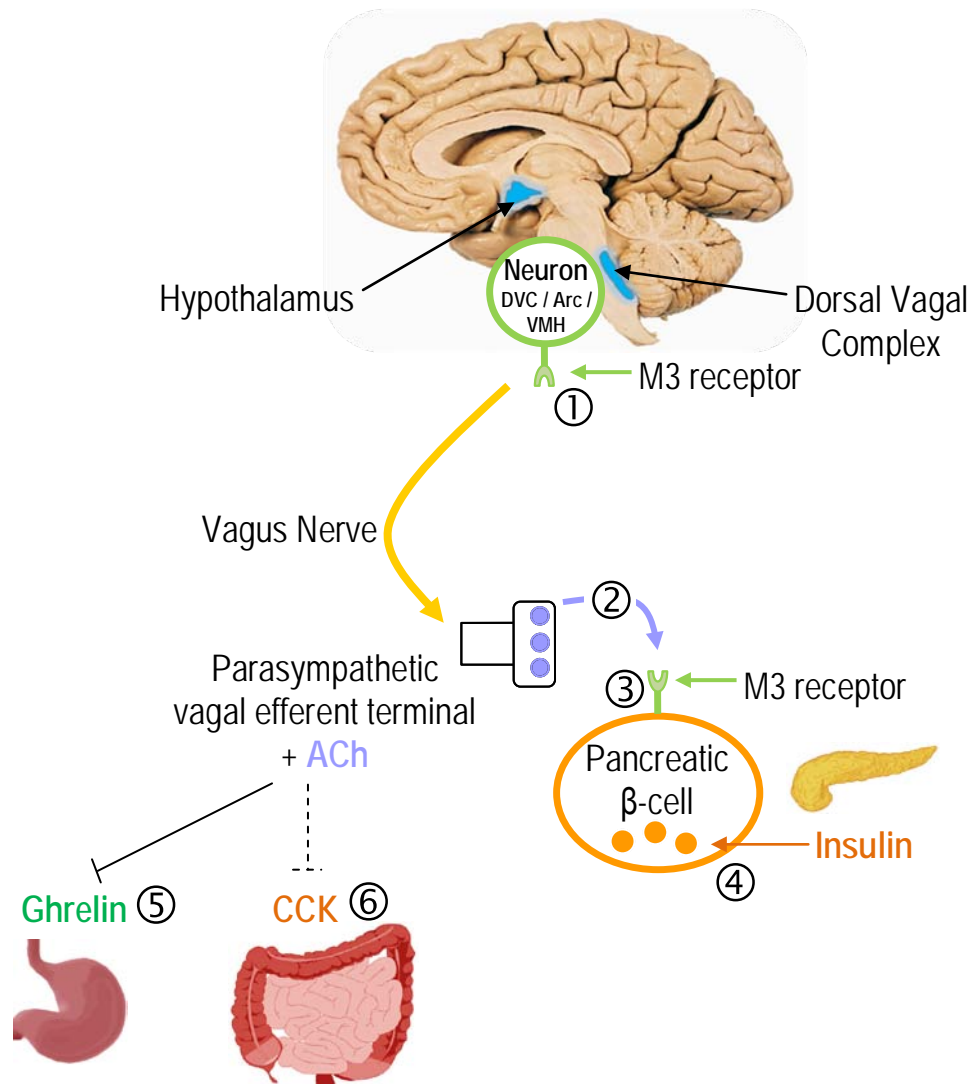


Figure 1.2: Potential Mechanism for the Involvement of Muscarinic M3 Receptors (M3Rs) in Olanzapine-Induced Metabolic Dysfunction

(1) Activation of the M3R in the hypothalamus and brainstem stimulates acetylcholine (ACh) release from the parasympathetic vagal efferent terminal (2). ACh activates M3Rs expressed on pancreatic β -cells (3) to facilitate the production and secretion of insulin (4). However, olanzapine is a potent M3R antagonist and blockade of the M3R may inhibit the cholinergic pathway for insulin secretion, resulting in hypoinsulinaemia following short-term treatment. M3R density may increase in response to M3R antagonism in an attempt to maintain homeostasis. This may cause a rise in insulin levels over time that precedes insulin-resistance and type II diabetes mellitus commonly observed in the clinic following chronic olanzapine treatment. (5) Olanzapine's blockade of M3R-mediated vagal-cholinergic innervation of the GI tract may also contribute, in-part, to its weight gain liability by altering the levels and action of other metabolic hormones, such as ghrelin (6) and CCK (7), in a manner that contributes to positive energy balance.

may result in a compensatory increase in insulin response in the long-term. In other words, M3R antagonism by olanzapine may disrupt the ACh-pathway for insulin secretion that precedes insulin-resistance and type II diabetes mellitus.

Olanzapine's antagonism of the M3R may also contribute to the pathogenesis of obesity through interference in the ACh-mediated regulation of gastrointestinal function, including secretion of hormones such as ghrelin and CCK, via vagal innervation to the stomach and intestines. For example, parasympathetic activity is low during periods of fasting to promote the gastric secretion of ghrelin (Lee et al., 2002). Ghrelin is tonically inhibited by the vagus nerve and truncal vagotomy results in an increase in circulating ghrelin levels, suggesting a cholinergic mechanism involving the DVC in the regulation of ghrelin secretion (Lee et al., 2002). ACh is the predominant excitatory neurotransmitter released from post-ganglionic terminals impinging on the GI tract (Travaglini et al., 2006). In addition, CCK secretion and satiating effects are influenced by vagal-cholinergic mechanisms (Liddle, 2000; Smith et al., 1981). Efferent preganglionic fibres originate in the DMN and afferent fibres from the GI tract feed into the DMN via the NTS. This vagal circuitry is sensitive to neurotransmitter signalling and input from other brain regions such as the hypothalamus. Therefore, direct drug antagonism of cholinergic receptors, for example the M3R, in these regions may impair metabolic signalling leading to an imbalance in energy homeostasis (Figure 1.2). However, the effects of olanzapine on M3Rs in regions of the brain that regulate insulin secretion and GI function are unknown.

b. Cannabinoid System: Role in Food Intake and Antipsychotic-Induced Weight Gain

The appetite enhancing effects of *cannabis sativa* (marijuana) were observed by Indian mendicants and utilised for the treatment of weight loss as far back as 300AD (Abel, 1975). Cannabinoids exert their effects through metabotropic $G\alpha_{i/o}$ -coupled receptors, termed CB1 (CB1R) and CB2 (CB2R). Cannabinoid receptors (CBRs) are expressed in areas such as the brain, pancreas, adipose tissue and skeletal muscle (Nogueiras et al., 2009) and on vagal afferent fibres innervating the GI tract (Burdyga et al., 2004; Harrold and Williams, 2003). The expression of CB1Rs in the brain is wide-spread, including the hypothalamus and particularly strong expression in the DVC (Glass et al., 1997; Van Sickle et al., 2003). Cannabinoid CB2Rs were initially thought to be confined to the peripheral immune system (Munro et al., 1993; Pertwee, 1997), however, later studies reported weak CB2R expression in the DVC (Van Sickle et al., 2005) and CB2R immunoreactivity in other brain regions (Gong et al., 2006).

Endogenous cannabinoids are important modulators of neural processes including cognition, neurodevelopment and central cardiovascular control (Bermudez-Silva et al., 2010). The cannabinoid system is also well-recognised for its influence on appetite. For example, exogenous cannabinoids, such as delta(9)-tetrahydrocannabinol (Δ^9 -THC, the main psychoactive component of marijuana) and CBR agonist, CP-55,940, increase food intake in rats and mice (Cota et al., 2003a; Miller et al., 2004; Wiley et al., 2005). In addition, endogenous cannabinoids, anandamide and 2-arachidonoyl glycerol (2-AG), promote re-feeding in satiated rats (Kirkham et al., 2002; Williams and Kirkham, 1999) and levels are increased in animal models of obesity, including obese *db/db* and *ob/ob* mice, and fatty Zucker rats (Di Marzo et al., 2001). The potential for the endocannabinoid system to modulate metabolism is vast, including central and peripheral implications in appetite, hedonic reward and addiction aspects of feeding, glucose and lipid

metabolism, and energy expenditure (Cavuto and Wittert, 2009; Harrold and Williams, 2003; Nogueiras et al., 2009). The hyperphagic effects of cannabinoids are thought to be mediated by the CB1R, for example, genetic CB1R-deficient mice are lean and hypophagic compared to their wild-type littermates (Cota et al., 2003b; Di Marzo et al., 2001; Wiley et al., 2005), and CB1R blockade by SR141716A (rimonabant hydrochloride) inhibits the hyperphagic effects of 2-AG, anandamide, CP-55,940 and Δ^9 -THC in rats and mice (Bermudez-Silva et al., 2010; Li et al., 2011). In fact, SR141716A was approved as a prescription drug for the treatment of human obesity in 2006, marketed as Acomplia® (Sanofi-Aventis, Paris, France), with reports of weight loss ranging from 5-10% of the initial body weight after 1 year of treatment (Van Gaal et al., 2005). However, it was suspended from the market in 2008 (Sanofi-Aventis press statement, Paris, November 5, 2008) following reports of serious adverse side-effects such as gastrointestinal dysfunction, mood disorder, depression and suicide (reviewed in Bermudez-Silva et al., 2010; Li et al., 2011; Van Gaal et al., 2005). SR141716A is a CB1R inverse agonist (i.e. it blocks potential agonist binding and inhibits receptor constitutive activation), however CB1R antagonism (i.e. partial or complete blockade of potential agonist binding) is still considered a viable potential pharmacological strategy against human obesity (Bermudez-Silva et al., 2010).

Endogenous cannabinoids are also important modulators of normal neuronal transmission. Unlike classical neurotransmitters, endocannabinoids can function as retrograde synaptic inhibitors of neuronal signalling (Alger, 2002) (Figure 1.3). They are released from the post-synaptic neuron and diffuse retrogradely across synapses to activate CB1Rs expressed on the presynaptic terminal. Upon activation, pre-synaptic CB1Rs suppress neurotransmitter release through a number of mechanisms, including inhibition of voltage-activated Ca^{2+} channels by the CB1R G-protein $\beta\gamma$ -subunits, which suppresses vesicular neurotransmitter release (Alger, 2002). Retrograde synaptic inhibition by the cannabinoid system has been reported in a number of brain

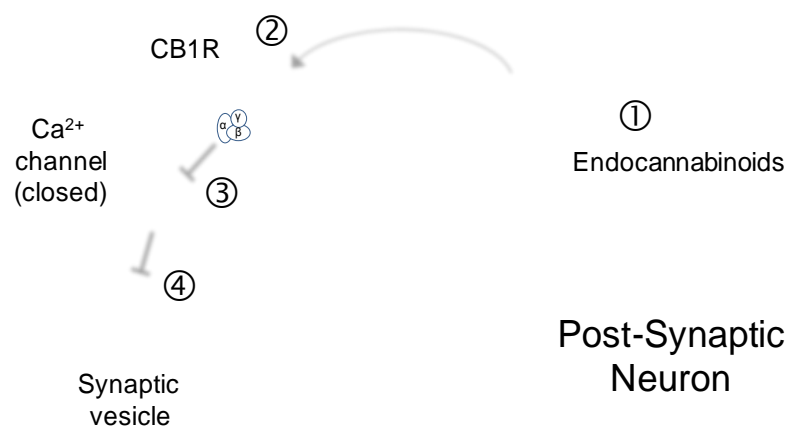


Figure 1.3: Schematic of Cannabinoid-Mediated Retrograde Transmission

(1) Endogenous cannabinoids are released from the post-synaptic cell either under basal conditions or following post-synaptic depolarisation. (2) Cannabinoids diffuse in a retrograde manner across the synaptic cleft and activate G protein-coupled cannabinoid CB1 receptors (CB1Rs) expressed by the pre-synaptic neuron or interneuron. (3) Activation of the CB1R releases the CB1R G protein-coupled $\beta\gamma$ sub-unit, which inhibits opening of the Ca^{2+} channel and influx of Ca^{2+} . (4) A reduction in Ca^{2+} decreases the probability of synaptic vesicle movement to the pre-synaptic cleft and subsequent release of the neurotransmitter onto the post-synaptic neuron.

regions, such as the hippocampus, striatum, cortex, and nucleus accumbens (Fukudome et al., 2004; Istvan et al., 1999; Kawamura et al., 2006; Narushima et al., 2006; Ohno-Shosaku et al., 2002; Sperl gh et al., 2009; Uchigashima et al., 2007; Urbanski et al., 2010), as well as the hypothalamus and DVC (Derbenev et al., 2004; Hentges et al., 2005; Ho et al., 2007; Nguyen and Wagner, 2007).

In the hypothalamus, POMC neurons of the Arc continuously express endocannabinoids under basal conditions that can activate CB1Rs on GABA interneurons and suppress GABA release (Hentges et al., 2005). In addition, Ho et al. (2007) reported a reduction in glutamatergic excitation of POMC by exogenous application of CB1R agonist, WIN 55,212-2, and an increase in glutamate firing on POMC following CB1R antagonism by AM251. These studies suggest a role for the cannabinoid system in regulating POMC activity in the hypothalamic Arc. As previously mentioned, the POMC gene is also expressed by neurons of the DVC (Bronstein et al., 1992), and CB1R-mediated retrograde inhibition of GABAergic and glutamatergic neurotransmission has been reported in the DVC. For example, Derbenev et al. (2004) reported presynaptic CB1R-mediated suppression of inhibitory and excitatory input to the DMN. They identified a number of DMN neurons as gastric-related, suggesting that cannabinoid signalling in the DVC may influence gastrointestinal function (Derbenev et al., 2004). Taken together, it is possible that interference in the normal signalling of the cannabinoid system may influence body weight as a result of altered GABAergic / glutamatergic input to POMC neurons in the hypothalamus and brainstem. Indeed, GABA is the predominant inhibitory neurotransmitter of the central nervous system and a role for GABAergic signalling in appetite regulation has been reported, i.e. hypothalamic GABA is increased in fasted rats during feeding (Meeker and Myers, 1980) and GABA is co-localised with both NPY and POMC neurons in the Arc (Meister, 2007). In addition, messenger RNA (mRNA) for glutamic acid decarboxylase (GAD) isoforms GAD₆₅ and GAD₆₇ (the rate limiting enzyme for

GABA synthesis), the vesicular GABA transporter and GABA transporter 1 proteins have been detected in the Arc and DVC (Meister, 2007; Stornetta and Guyenet, 1999; Yasumi et al., 1997). Anorexigenic PYY₍₃₋₃₆₎ decreases GABA release on POMC neurons (Batterham et al., 2002), and GABA mediates NPY tonic inhibition of POMC in the Arc (Cowley et al., 2001). Therefore, it is possible that GABAergic signalling in the hypothalamus may be involved in antipsychotic-induced weight gain side-effects.

i. Cannabinoid Neurotransmission and Antipsychotic Drugs: Role in Metabolic Dysfunction

We have previously shown that olanzapine treatment induces a robust decrease in [³H] CP-55,940 binding density in the DVC of rats, whereas low metabolic-risk antipsychotics, aripiprazole and haloperidol, have little or no effect (Weston-Green et al., 2008). In addition, we observed a strong positive correlation between [³H] CP-55,940 binding density and body weight gain following chronic olanzapine treatment (Weston-Green et al., 2008). An olanzapine-induced decrease in CBR binding density during body weight gain does not appear to fit with the documented appetite and body weight suppressive effects of CB1R blockade (Li et al., 2011). However, interference in cannabinoid signalling by olanzapine may affect anorexigenic POMC and contribute to body weight gain side-effects. As mentioned in the section above, endogenous cannabinoids released from POMC neurons in the Arc activate CB1Rs expressed on GABA neurons and can suppress GABAergic inhibition of the post-synaptic POMC neuron (Hentges et al., 2005; Ho et al., 2007; Nguyen and Wagner, 2007). Reduced GABA may disinhibit POMC and promote hypophagia (Ho et al., 2007). A reduction in CB1R binding density following olanzapine treatment may decrease CB1R-mediated suppression of GABA, reinstating GABAergic inhibition of POMC. Therefore, it is possible that olanzapine-induced weight gain side-effects occur, in part, through reduced anorexigenic POMC expression in the Arc and DVC as a result of decreased

CB1R-mediated retrograde inhibition of GABA. However, the effects of olanzapine on CB1R in the hypothalamus are unknown, and olanzapine's influence on POMC and GABA expression in these regions is unclear. In addition, the ligand used in our earlier study, CP-55,940, has a high affinity for the CB1R, but also binds at a low level to the CB2R (Pertwee, 1997), therefore, whether the decrease in CBR binding density observed in the DVC following olanzapine treatment could be attributed to the CB1R requires clarification.

1.2.4 Animal Model of Antipsychotic-Induced Weight Gain

Clearly there are many questions about the mechanisms by which SGAs alter appetite and body weight regulation. However, the ability to investigate antipsychotic effects in humans *in-vivo*, particularly in the brain, is limited for obvious technological and ethical reasons. An appropriate animal model that closely mimics the human scenario is required in order to examine the central mechanisms of antipsychotic-induced metabolic dysfunction. For the past decade scientists have worked to establish an animal model that mimics human antipsychotic-induced metabolic side-effects. However, reports are varied possibly due to differences in experimental parameters such as drug selection, dosage, and method of drug administration, treatment duration and interval, and strain and gender of animal. The experimental parameters and outcomes of a large number of animal modelling studies of antipsychotic-induced metabolic dysfunction were recently reviewed (Boyda et al., 2010a; Coccurello and Moles, 2010).

A. Antipsychotic Drug Selection, Dosage Regime and Administration Method

Although clinical data indicates clozapine has the highest metabolic liability of all antipsychotic drugs (Allison et al., 1999; Newcomer, 2005), reports to-date have shown limited success in modelling clozapine-induced obesity side-effects in the rat or mouse (Choi et al., 2007; Coccurello and Moles, 2010; Cooper et al., 2008a). In fact, clozapine treatment can even reduce

body weight in the rat (Cooper et al., 2008a). This failure to induce weight gain or hyperphagia in rats treated with clozapine may be due to sedation that interferes with eating behaviour (Albaugh et al., 2006; Cooper et al., 2008a). Some studies have been able to replicate certain aspects of clinically-reported metabolic side-effects in the rat using clozapine, for example, hyperglycaemia, hyperinsulinaemia, impaired insulin sensitivity and induced insulin-resistance (Boyda et al., 2010b; Houseknecht et al., 2006; Murashita et al., 2007; Smith et al., 2008; Tulipano et al., 2007), enhanced fat intake (Hartfield et al., 2003), increased ghrelin and adiponectin levels (Cooper et al., 2008a; Murashita et al., 2007), and enhanced adipose accumulation; though the latter had no effect on overall body weight (Cooper et al., 2008a). Other studies have reported on the effects of risperidone, quetiapine, ziprasidone and sulpiride on metabolic parameters in rats and mice with varied outcomes (Baptista et al., 2002; Boyda et al., 2010a; reviewed in Coccurello and Moles, 2010; Cope et al., 2009; Ota et al., 2002; Savoy et al., 2008; Smith et al., 2008), though these drugs have a lower clinical metabolic risk than olanzapine and clozapine (Allison et al., 1999; Newcomer, 2005).

Multiple aspects of clinically-reported metabolic side-effects following olanzapine treatment have been modelled in rats and mice, including body weight gain, hyperphagia, adiposity, hypolocomotor activity, hyperglycaemia, hyperinsulinaemia, insulin-resistance, and circulating metabolic hormone imbalances (Albaugh et al., 2010; Baptista et al., 2007; Beebe et al., 2006; Boyda et al., 2010b; Chintoh et al., 2009; Chintoh et al., 2008a; Coccurello et al., 2009; Coccurello et al., 2008; Cooper et al., 2008a; Huang et al., 2006b; Lee and Clifton, 2002; Lykkegaard et al., 2008; Park et al., 2010; Shobo et al., 2010; van der Zwaal et al., 2010), (also see Coccurello and Moles, 2010 for review). However, animal modelling studies differ in numerous experimental design parameters, including dosage. For example, olanzapine increased food intake, weight gain, adiposity and induces insulin-resistance in female Han-Wistar, Hooded-

Lister and Sprague Dawley (SD) rats at dosages ranging from 0.5 – 8mg/kg/day (Albaugh et al., 2006; Arjona et al., 2004; Choi et al., 2007; Cooper et al., 2005; Fell et al., 2004; Pouzet et al., 2003), while 20mg olanzapine treatment had no effect on food intake or weight gain (Albaugh et al., 2006; Pouzet et al., 2003). In order to resemble clinical antipsychotic-induced metabolic dysfunction, dosage selection for use in an animal model should reflect a clinically-relevant amount, i.e. the recommended olanzapine dose in humans is 5-20mg/day, or 0.07mg-0.31mg/kg/day for the average 65kg individual (Kapur et al., 2003). However, direct dosage translation between humans and rats based on body weight may be inappropriate due to differences in drug absorption, distribution and metabolism (Kapur et al., 2003). Pharmacokinetic differences are even noted between rat species (Kapur et al., 2003). A widely accepted measure of dosage translation is based on clinically comparable dopamine D2 receptor occupancy levels (Kapur, 1998; Kapur et al., 2003), and calculating differences in body surface area between species (Reagan-Shaw et al., 2007). Another consideration is treatment administration, which can alter drug uptake *in-vivo*, for example, mini-pump administration of olanzapine results in a D2 receptor occupancy of up to 2 times higher than the same dosage administered by subcutaneous injection (Kapur et al., 2003), and oral gavage can result in more than 2-times higher concentration of olanzapine in the brain than intra-peritoneal injection (Aravagiri et al., 1999). In addition, the half-life of olanzapine is approximately 75.2 hours in the human brain (Tauscher et al., 2002), whereas the drug metabolises at a much faster rate in the rat, i.e. olanzapine has a half-life of 5.1 hours in the rat brain though high levels remain after 8-hours (Aravagiri et al., 1999). Therefore, multiple dosages may be required in the rat (8-hourly intervals, three times daily (t.i.d.) may be required in order to minimise drug fluctuations below sub-therapeutic D2 receptor occupancy levels (Kapur et al., 2003). One method of overcoming inappropriate oscillations in drug levels may be through the use of a mini-pump (Kapur et al., 2003), however, issues such as intolerable acidification of solvent in order to achieve the correct drug

concentration (Kapur et al., 2003) and degradation of olanzapine in solution leading to unstable levels (van der Zwaal et al., 2008) have been reported, particularly past 14-days' treatment (Remington et al., 2011). Other reported treatment administration methods include subcutaneous or intraperitoneal injection, or oral gavage (Arjona et al., 2004; Fell et al., 2004; Pouzet et al., 2003), which involve handling and potential injury (de Meijer et al., 2010) and stress on the animal (Balcombe et al., 2004). A review of 80 studies revealed that physical handling of rats and mice, including moving their cages, can increase active behaviour (reports range from +190% to +441%), body temperature (+2.7%), glucose (reports range from +1% to +195%) and growth hormone (+400%), as well as numerous other parameters, such as heart rate and blood pressure (Balcombe et al., 2004), that may affect the outcome of studies pertaining to food intake and weight gain. Oral self-administration through drinking water has been reported (Gao et al., 2005; Raskind et al., 2006; Shertzer et al., 2010), however maintaining consistent dosing may be problematic as the majority of antipsychotic drugs are not readily soluble in water, particularly olanzapine (Eli Lilly and Company Material Data Safety Sheet, Zyprexa Tablets, v1.3, 04/07/2011). Another method of drug delivery is oral self-administration and positive reinforcement through a palatable reward, eg: chocolate, peanut butter, or cookie-dough (Cope et al., 2009; Han et al., 2008a; Huang-Brown and Guhad, 2002). Several studies have reported sensitivity to metabolic side-effects in rats orally self-administered olanzapine in their food (Minet-Ringuet et al., 2006b; Shobo et al., 2010), even enhanced response compared to gavage or injection methods (Minet-Ringuet et al., 2006b). A number of other studies have successfully modelled antipsychotic-induced metabolic side-effects using a cookie-dough administration method whereby animals learn to eat a sweet-tasting 'cookie' containing the required drug dosage (Albaugh et al., 2006; Albaugh et al., 2010; Deng et al., 2007b; Han et al., 2008a; Huang-Brown and Guhad, 2002). The method of oral self-administration mirrors the clinic, is less invasive than mini-pump implants, and may alleviate handling stress and risk of injury to the

animal associated with oral gavage and injection. In addition, orally self-administered multiple dosing in 24 hours may allow a consistently high drug dosage in the rat.

B. Gender Differences

Parts of the following section were published in Weston Green et al., (2010). Sensitivity of the Female Rat to Olanzapine-Induced Weight Gain – Far from the Clinic?, *Schizophrenia Research* 116(2-3): 299-300 (Appendix I).

One of the issues overshadowing the ability of the olanzapine-treated animal model to resemble the clinic is the reported difficulty in replicating olanzapine-induced metabolic dysfunction in the male rat (Albaugh et al., 2006; Choi et al., 2007; Minet-Ringuet et al., 2006b; Minet-Ringuet et al., 2005; Pouzet et al., 2003). However, several studies have reported success in modelling olanzapine-induced metabolic dysfunction in male rats under certain conditions (Hartfield et al., 2003; Minet-Ringuet et al., 2006a; Ota et al., 2002; Shobo et al., 2010; Thornton-Jones et al., 2002; van der Zwaal et al., 2010). In addition, a growing body of evidence suggests that female individuals with schizophrenia are more sensitive to the metabolic side-effects of antipsychotic drugs than males (Bobes et al., 2003; Hakko et al., 2006; Seeman, 2010; Wu et al., 2007). This suggests that the sensitivity of the female rat to SGA-induced metabolic dysfunction may not be as far from the clinic as previously believed.

Based on clinical data, we suggest that the rodent model of olanzapine-induced weight gain mimics aspects of the human situation as studies have revealed gender-related differences in the human response to olanzapine treatment. Evidence shows that females with a psychotic disorder have a 3.6-fold increased risk of weight gain than males (Hakko et al., 2006) and previous studies have identified female gender as a risk factor and predictor for weight gain associated with

olanzapine and other atypical antipsychotics (Gebhardt et al., 2009). In fact, Wu et al. (2007) reported that female first-episode schizophrenia patients had a higher hip to waist ratio, increased insulin-resistance and higher plasma triglycerides than males following treatment with olanzapine and clozapine. Kluge et al. (2009) found that olanzapine significantly increased the BMI of female patients after 1 week of treatment, however male patients took longer to reach significance, and females exhibited increased skin-fold thickness but not males. Females also showed a 2–4 times higher level of plasma leptin than males following olanzapine treatment, and this increase was observed earlier in females than in male patients (Kluge et al., 2009). Furthermore, female schizophrenia patients are more responsive to olanzapine treatment than male patients, regardless of illness chronicity (Usall et al., 2007). Female patients also exhibit higher plasma concentrations of the drug than males (Kelly et al., 1999), possibly due to their generally lower lean body mass and increased adipose tissue, allowing greater drug storage and leading to higher plasma levels over time (Yonkers et al., 1992). Gonadal steroids such as oestrogen, progesterone and testosterone can influence food intake and metabolism. Fitzgerald et al. (2003) identified significant positive correlations between changes in oestrogen levels and alterations in leptin and NPY levels as well as BMI and weight gain in female schizophrenia patients treated with olanzapine or risperidone. These results suggested that fluctuating gonadal steroid levels may play a role in the weight gain side-effect of atypical antipsychotic drugs and may explain the higher sensitivity of females to antipsychotic-induced weight gain, though the exact mechanism is unknown (Fitzgerald et al., 2003). Finally, based on waist circumference measurements, olanzapine-treated male patients are more responsive to nutritional intervention than females (Skouroliaou et al., 2009). Indeed, human studies have shown that atypical antipsychotic-induced weight gain does not occur in all patients, and findings from the large-scale Clinical Antipsychotic Trials of Intervention Effectiveness study showed an increase of 7% weight gain from baseline in 30% of patients treated with olanzapine (Allison et al., 2009). Taken together, the

rodent model olanzapine-induced weight gain cannot completely replicate the human weight gain side-effect, particularly in male rats. However, the sensitivity of female rodents to this side-effect over males appears to be a common observation in the clinic. Future studies on sex differences in the rodent model may improve our understanding of the mechanisms underlying gender response to antipsychotic effects.

C. Overall Comments on Animal Models of Antipsychotic-Induced Weight Gain

Overall, modelling metabolic dysfunction in rats is sensitive to the antipsychotic studied, dosage, treatment interval, method of administration, and gender of the animal. Olanzapine-induced metabolic dysfunction can be modelled in female rats; however the most appropriate dosage that best mimics human metabolic dysfunction side-effects is unclear. Voluntary oral self-administration resembles the clinic and may reduce animal stress associated with handling. Based on the half-life of olanzapine, 8-hourly drug treatment intervals are required in order to maintain appropriate drug levels in the brain. Female rats appear to be more sensitive to antipsychotic-induced weight gain, which does resemble reports from the clinic (Bobes et al., 2003; Seeman, 2010).

An appropriate animal model of olanzapine-induced metabolic dysfunction will allow examination of drug-induced metabolic changes, including investigation of central mechanisms *in-vivo* that cannot be explored in humans. In addition, a reliable animal model of olanzapine-induced metabolic dysfunction is essential for the pre-clinical development of therapeutic interventions aimed at attenuating or preventing metabolic side-effects.

1.3 AIMS

1.3.1 General Aim

To examine the molecular effects of olanzapine, a second-generation atypical antipsychotic drug with a high metabolic liability, on key neurotransmitter signalling systems in the hypothalamus and brainstem, in order to elucidate the mechanisms underlying olanzapine-induced metabolic side-effects.

1.3.2 Specific Aims

The specific aims of this research were to:

1. Validate a clinically-relevant animal model of olanzapine-induced metabolic dysfunction by examining drug dosage effects on body weight, food and water intake, subcutaneous and visceral adiposity, locomotor activity and circulating metabolic hormones insulin, ghrelin, CCK and PYY₍₃₋₃₆₎ in the female rat after sub-chronic (14-days) treatment;
2. Investigate the dosage effects of olanzapine on muscarinic M3R binding density in the hypothalamus and brainstem, and examine the relationship between M3R binding density and body weight, food intake, plasma insulin, ghrelin, CCK and glucose;
3. Investigate the dosage effects of olanzapine on POMC and NPY mRNA expression, cannabinoid CB1R binding density, and GABA synthesis (via GAD mRNA expression) in the hypothalamus and brainstem, and to examine their relationship to body weight gain and visceral adiposity

1.3.3 Hypotheses

1. Similar to reports in the clinic, olanzapine will induce body weight gain, accompanied by hyperphagia, increased adiposity, reduced locomotor activity and metabolic hormone alterations in the female rat
2. Based on the binding profile of olanzapine, the M3R will be effected by drug treatment and these alterations will correlate to metabolic dysfunction side-effects
3. Olanzapine will alter POMC and NPY mRNA expression, cannabinoid CB1R binding density, and GAD mRNA expression and these changes will correlate to body weight and visceral adiposity.
4. There will be a dose-sensitive response in the parameters measured in the present study, with a general trend for amplified response with increased dosage within the dosage range tested.

1.3.4 Significance

Despite significant improvements over the past 60 years in psychopharmacological therapies for the treatment of schizophrenia and other psychotic illnesses, some widely prescribed antipsychotics are still associated with intolerable side-effects, including obesity and type II diabetes mellitus. Antipsychotic-induced metabolic side-effects are particularly concerning due to associated co-morbidities, including cardiovascular disease and stroke, and patient non-compliance. In addition, the incidence of obesity and diabetes is 1.5-2.0 times higher in individuals with schizophrenia than the general population, highlighting the urgency of discovering new drug strategies with high efficacy and low risk of adverse side-effects. In order to assist the development of new antipsychotics with low metabolic liability, an understanding of the mechanisms underlying antipsychotic-induced metabolic dysfunction is required. Reports over the past decade have offered a number of hypotheses on the mechanisms of antipsychotic-induced

metabolic side-effects, however the answer remains unclear. Indeed, based on the broad receptor binding profile, it is likely that a number of metabolic alterations may combine to manifest antipsychotic metabolic side-effects.

Exploring the effects of olanzapine, a high metabolic risk antipsychotic drug, on candidate neurotransmitter signalling systems in regions of the brain that regulate energy homeostasis will provide novel understanding of the mechanisms underlying the metabolic side-effects. Results from the present study may provide direction that can underpin the design and facilitation of clinical trials aimed at improving antipsychotic-induced metabolic dysfunction in order to enhance patient outcomes and restore quality of life. In addition, validation of a clinically-relevant animal model of olanzapine-induced metabolic dysfunction will allow *in-vivo* investigation of these side-effects and assist the experimental design of future animal model studies, particularly with respect to olanzapine dosage selection.

1.4 GENERAL METHODS

1.4.1 Ethics Statement

This study was approved by the Animal Ethics Committee, University of Wollongong (Application Approval #: AE06/32), and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004), which is in accordance with the International Guiding Principles for Biomedical Research Involving Animals. All efforts were made to minimise animal stress and prevent suffering.

1.4.2 Animals and Drug Treatment

Sixty female adult (7 week old) Sprague Dawley rats were obtained from the Animal Resource Centre (Perth, WA, Australia) and were housed individually under a 12-hour light-dark cycle (photophase: 07.00h) at 22°C, with *ad libitum* access to water and standard laboratory chow (3.9 kcal/g; 10% fat, 74% carbohydrate, 16% protein) throughout the study. Upon arrival, animals were randomly assigned to 0.25mg/kg, 0.5mg/kg, 1.0mg/kg or 2.0mg/kg olanzapine treatment groups, or the control group (n=12/group). Animals were habituated for 1 week, then underwent a teaching period of 1-week where they were taught to self-administer a sweet cookie-dough pellet offered by metal spoon and were sham-weighed to minimise handling stress during the treatment period. Methods of olanzapine treatment via cookie dough self-administration were modified from Huang-Brown and Guad (2002), as previously described by our laboratory (Han et al., 2008a; Han et al., 2008b; Huang et al., 2006a). Briefly, dry ingredients containing cornstarch (30.9%), sucrose (30.9%), gelatine (6.3%), casein (15.5%), fibre (6.4%), minerals (8.4%) and vitamins (1.6%) (62% carbohydrate, 22% protein, 6% fibre, 10% vitamins and minerals) were mixed. Olanzapine tablets were pulverised using a mortar and pestle following removal of tablet coating. The calculated dosage of powdered olanzapine was added to weighed dry ingredients and

enough drops of distilled water were added to achieve a dry dough consistency, immediately prior to administration. Animals were treated with 0.3g cookie-dough pellets with the assigned olanzapine dosage (or control) offered by metal spoon three-times daily (t.i.d.) at 8-hourly intervals for 14 days. Consumption of each pellet was observed to ensure successful dosing. Body weight, and food and water intake (corrected for spillage) were measured daily.

1.4.3 Behavioural Analysis: Open Field Testing

Animals were subjected to open field behavioural testing on treatment days 8-10. Methods were employed as previously described by our laboratory (du Bois et al., 2008). A single animal was placed in the centre of a 60 cm x 60 cm wide black square arena with 40 cm high walls exposed to a light intensity of 100 lux. Animal behaviour (distance travelled, velocity, rearing frequency, and central and peripheral duration) was recorded for 30-minutes and analysed using Ethovision video-tracking software (Nodulus Information Technology, Wageningen, The Netherlands) (Figure 1.4).

1.4.4 Hormonal and Adipose Measurements

Following 4-6 hours fasting, animals were euthanized 10-12 hours from the last drug treatment using sodium pentobarbitone. Blood was removed from the left ventricle. Blood samples for each hormone were separately collected in tubes containing 2Na-EDTA, aprotinin, K₃EDTA or heparin and immediately centrifuged. Plasma was aliquoted and stored at -20°C. Commercially available radioimmunoassay (RIA) kits were used to measure plasma CCK, PYY₍₃₋₃₆₎ (Phoenix Pharmaceuticals, CA, USA) and total ghrelin (Linco Research, MI, USA), while insulin was detected using enzyme-linked immunosorbent assay (ELISA) kits (see Chapters 2 and 3 for details). Adiposity was measured by individually dissecting and weighing inguinal, perirenal and periovary white fat pads, and sub-scapula brown fat pads.

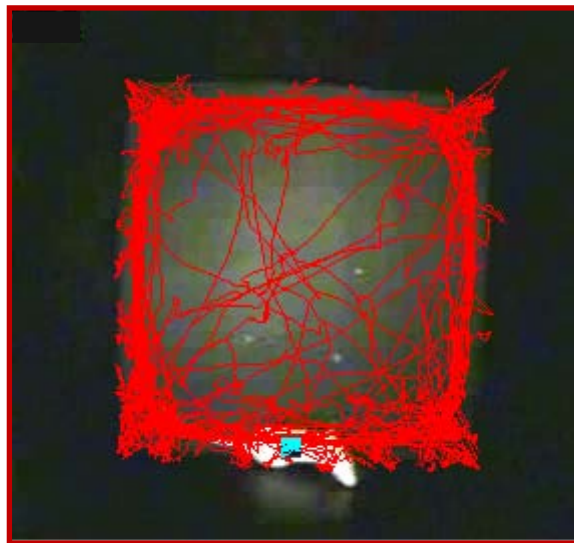


Figure 1.4: Example of Open-Field Testing and Ethovision Trace

An example of open-field testing used to examine locomotor activity in a female Sprague Dawley rat. Animal was placed in the centre of a 60 cm x 60 cm wide black square arena with 40cm high walls exposed to a light intensity of 100 lux. Locomotor activity (i.e. distance travelled, velocity, rearing frequency, and central and peripheral duration) was recorded for 30-minutes. The red line is a trace of the animal's movement, analysed using Ethovision video-tracking software (Nodulus Information Technology, Wageningen, The Netherlands).

1.4.5 Histology

Brain tissue was immediately removed following euthanasia, frozen in liquid nitrogen then stored at -20°C. Six brains were randomly selected from each treatment group and cryostatally sectioned (14µm, -18°C) along the coronal plane at the levels of Bregma incorporating the hypothalamus and the DVC (Paxinos and Watson, 2007) (Figure 1.5). Sections were thaw-mounted onto Polysine™ Microscope Slides (Menzel GmbH & Co. KG, Braunschweig, Germany) and stored at -20°C.

1.4.6 Receptor Binding

Muscarinic M3 and cannabinoid CB1 receptor binding density experiments were performed using methods detailed in Chapters 3 and 4, respectively. Radiographic images were captured using a Beta Imager camera (BioSpace, Paris, France). Radioactivity levels were counted from the amount of β -particles emitted from the brain sections for 3.5 hours. A slide containing sections with a known amount of tritium [^3H] was used to construct a standard curve. β -Imager Plus software (version 4, BioSpace) was used to quantify radioactive levels in the hypothalamic Arc and VMH, and the DVC of the brainstem. The position of the nuclei of interest were confirmed using a set of cresyl violet-stained slides and a rat brain atlas (Paxinos and Watson, 2007). Radioligand binding density measurements were converted from nCi/mg tissue to fmol/mg tissue equivalent using the aforementioned standards.

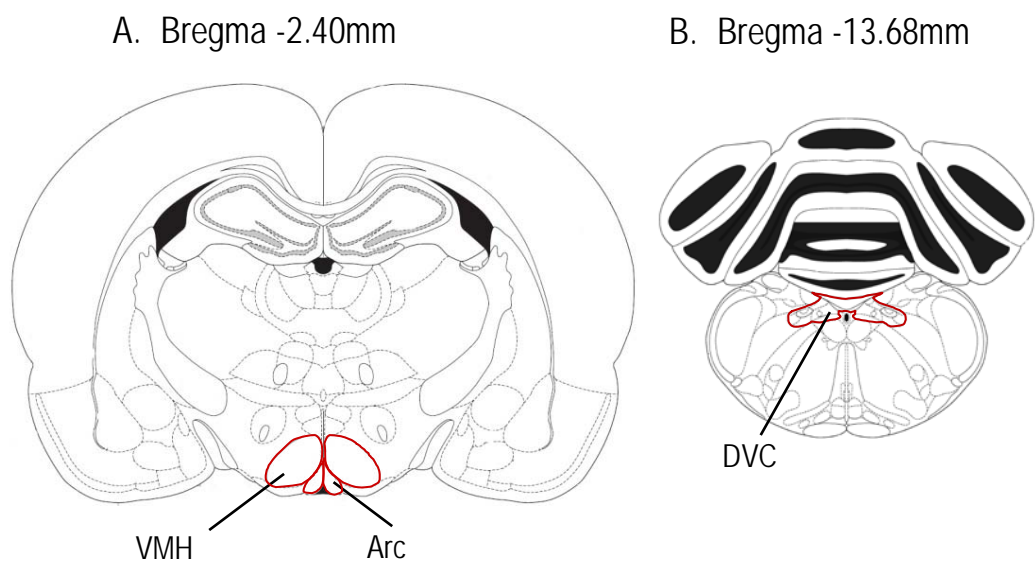


Figure 1.5: Schematic of the rat brain

Depicts the levels of Bregma (A) -2.40mm, incorporating the hypothalamic ventromedial nucleus (VMH) and arcuate nucleus (Arc); (B) -13.68mm, incorporating the dorsal vagal complex of the caudal brainstem. Modified from Paxinos and Watson (2007). *The Rat Brain*. 6th Ed. Academic Press Inc, USA.

1.4.7 *In-situ* Hybridisation

POMC, GAD₆₅ and NPY mRNA expression methodology is described in Chapters 3 and 4. Expression of mRNA was performed by terminally labelling specific antisense hybridisation probes with [³⁵S]dATP (1000 Ci/mmol, Perkin Elmer, Waltham, MA, USA) in 10-fold molar excess, and terminal transferase (Promega, Madison, WI, USA). Labelled probes were purified using a MicroSpin G-50 column (GE Healthcare Ltd, Buckinghamshire, UK). Slides were incubated in hybridisation buffer containing 4×SSC, 1×Denhardt's solution, 50% de-ionised formamide, 200 µg/ml sperm DNA, 100 µg/1 ml polyA, 120 µg /ml heparin, 20 mM sodium phosphate and labelled probe (18-hours, 37°C, pH 7.0). Slides were washed in 1x SSC buffer (3 x 30-minutes, 55°C) and incubated in SSC buffer (1-hour, room temperature). Sections were sequentially dipped in Milli-Q water, 70% then 95% ethanol, and dried under a gentle stream of air. Autoradiographic images were captured on film (Kodak BioMax MR film, Rochester, NY, USA). Following exposure for 3 weeks, films were developed using standard procedures. Slides containing sections with positive signals were then dipped in Emulsion solution (GE Healthcare Ltd, Buckinghamshire, UK), and exposed for a further 6 weeks. Emulsified slides were stained using cresyl violet for further identification of positive signals at a cellular level.

Films were analysed using a computer-assisted image analysis system, Quantity One (v4.6.7, Bio-Rad Laboratories, Inc, CA, USA), connected to a GS-800 Densitometer (Bio-Rad Laboratories, Inc). Autoradiographic standards containing a known amount of radioactive carbon [¹⁴C] (GE Healthcare Ltd, Buckinghamshire, UK) were used to generate a standard curve.

1.4.8 Statistical Analysis

Power calculation analysis during experimental design revealed a power of 80-85% (JMP 5.1, SAS Institute Inc., USA), based on studies by Arjona et al. (2004) and Huang et al. (2006a). For a

power of 80%, 12 animals per group were required to examine body weight, food intake and behaviour, whereas 6 animals per group were required for neurochemical analysis, in order for results to be significantly different at an alpha level of 0.05.

All remaining statistical analysis was performed using SPSS (Chicago, IL, USA, version 17.0). Data points that were outside ± 2 standard deviations from the mean were excluded from analysis. Data was tested for normal distribution using Kolmogorov-Smirnov Tests. Two-way repeated analysis of variance (ANOVA) tests were employed ('dosage' x 'time' (days) as a repeated factor) to examine body weight, food and water intake. Plasma hormones levels, adiposity, behavioural data, receptor binding density and *in-situ* hybridisation data were analysed using one-way ANOVAs. Post-hoc analysis was performed using Dunnett-T tests (when comparing treatment groups against the control) or Tukey HSD tests (when comparing treatment groups against the control and other treatment groups). A non-parametric Kruskal-Wallis test was followed by Mann-Whitney U post-hoc analysis in order to analyse total ghrelin. Throughout the present study, correlations were observed using Pearson's correlation tests, or Spearman's tests for non-parametric data. Data is expressed as mean \pm S.E.M. A *p*-value less than 0.05 was considered statistically significant.

1.5 THESIS SUMMARY

The mechanisms underlying olanzapine-induced metabolic dysfunction in humans remain unclear. Clinical studies have shown that antipsychotic treatment can interfere with the levels of circulating metabolic hormones, such as ghrelin and insulin; however the results are conflicting. Circulating metabolic hormones exert their effects on the brain, particularly the hypothalamus and caudal brainstem, to signal the energy status of the body. The literature has revealed the importance of the muscarinic, melanocortinergeric, GABA and cannabinoid neurotransmission systems in regulating appetite, glycaemia and body weight. Alterations to these neurotransmitter signalling systems during olanzapine treatment may favour a positive energy state and contribute to body weight gain and insulin dysregulation side-effects. However, studies on the effects of olanzapine on each of these systems in the hypothalamus and brainstem are limited or lacking. The ability to examine alterations to neurotransmitter signals during olanzapine treatment is limited in the human *in-vivo*. Olanzapine-induced metabolic dysfunction can be modelled in the rat; however the appropriate dose to use is unclear. Therefore, it is necessary to validate a clinically comparable animal model of olanzapine-induced metabolic side-effects and identify the minimum dosage threshold required to change the above-mentioned parameters.

The main findings of the present thesis are summarised in Table 5.1, page 94. Summary abstracts from each study are shown in the following Sections (1.5.1- 1.5.3).

1.5.1 Olanzapine Treatment and Metabolic Dysfunction: A Dose Response Study in Female Sprague Dawley Rats

SGAs are commonly prescribed for the treatment of schizophrenia, however some can induce metabolic dysfunction side-effects such as weight gain, obesity and diabetes. Clinical reports suggest olanzapine alters satiety signals, although findings appear conflicting. Previous animal model studies have utilised a range of olanzapine dosages, however the dosage that better mimics the human scenario of olanzapine-induced weight gain is unclear. Female Sprague Dawley rats were treated orally, three times daily with olanzapine (0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg), self-administered in a sweet cookie dough pellet at eight-hourly intervals) or vehicle (n=12/group) for 14 days. Olanzapine orally self-administered in multiple doses (eight-hourly intervals) may circumvent a drop in plasma drug concentration and ensure the maintenance of a consistently high olanzapine level in the rat. Olanzapine increased body weight (0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg), food intake (2.0 mg/kg) and feeding efficiency (0.5–2.0 mg/kg), with no effect on water intake. Subcutaneous inguinal (1.0 mg/kg, 2.0 mg/kg) and intra-abdominal perirenal fat were increased (2.0 mg/kg), but not interscapula brown adipose tissue. Olanzapine increased circulating ghrelin and cholecystokinin, but had no effect on peptide YY_(3–36). Olanzapine decreased insulin (0.25–2.0mg/kg) and locomotor activity in the open field arena (0.5–2.0 mg/kg). A low dosage of 0.25 mg/kg olanzapine had no effect on most parameters measured. Olanzapine-induced weight gain is associated with hyperphagia, enhanced feeding efficiency and adiposity, decreased locomotor activity and altered satiety signalling. The animal model used in the present study of self-administered oral olanzapine treatment (t.i.d.) at a dosage range of 0.5–2.0 mg/kg (but not 0.25 mg/kg) mimics aspects of the clinic.

1.5.2 Effects of olanzapine on muscarinic M3 receptor binding density in the brain relates to weight gain, plasma insulin and metabolic hormone levels

Cholinergic muscarinic M3 receptors (M3R) are expressed on pancreatic β -cells and in the brain where they influence insulin secretion and may regulate other metabolic hormones via vagal innervation of the gastrointestinal tract. Olanzapine's M3R antagonism is an important risk factor for its diabetogenic liability. However, the effects of olanzapine on central M3Rs are unknown. Rats were treated with 0.25, 0.5, 1.0 or 2.0 mg olanzapine/kg or vehicle (3x/day, 14 days). M3R binding densities in the hypothalamic arcuate (Arc) and ventromedial nuclei (VMH), and dorsal vagal complex (DVC) of the brainstem were investigated using [3 H]4-DAMP plus pirenzepine and AF-DX116. M3R binding correlations to body weight, food intake, insulin, ghrelin and cholecystokinin (CCK) were analysed. Olanzapine increased M3R binding density in the Arc, VMH and DVC, body weight, food intake, circulating plasma ghrelin and CCK levels, and decreased plasma insulin and glucose. M3R negatively correlated to insulin, and positively correlated to ghrelin, CCK, food intake and body weight. Increased M3R density is a compensatory up-regulation in response to olanzapine's M3R antagonism. Olanzapine acts on M3R in regions of the brain that control food intake and insulin secretion. Olanzapine's M3R blockade in the brain may inhibit the acetylcholine pathway for insulin secretion. These findings support a role for M3Rs in the modulation of insulin, ghrelin and CCK via the vagus nerve and provide a mechanism for olanzapine's diabetogenic and weight gain liability.

1.5.3 Alterations to Melanocortinergic, GABAergic and Cannabinoid Neurotransmission Associated with Olanzapine-Induced Weight Gain

This study examined the effects of low to high doses of olanzapine on appetite/metabolic regulatory signals in the hypothalamus and brainstem to elucidate the mechanisms underlying olanzapine-induced obesity. Levels of pro-opiomelanocortin (POMC), neuropeptide Y (NPY) and glutamic acid decarboxylase (GAD₆₅, enzyme for GABA synthesis) mRNA expression, and cannabinoid CB1 receptor (CB1R) binding density (using [³H]SR-141716A) were examined in the arcuate nucleus (Arc) and dorsal vagal complex (DVC) of female Sprague Dawley rats following 0.25, 0.5, 1.0 or 2.0mg/kg olanzapine or vehicle (3x/day, 14 days). Consistent with its weight gain liability, olanzapine significantly decreased anorexigenic POMC and increased orexigenic NPY mRNA expression in a dose-sensitive manner in the Arc. GAD₆₅ mRNA expression increased and CB1R binding density decreased in the Arc and DVC. Alterations to neurotransmission signals in the brain significantly correlated with body weight and adiposity. The minimum dosage threshold required to induce weight gain in the rat was 0.5mg/kg olanzapine. Olanzapine-induced weight gain is associated with reduced appetite-inhibiting POMC and increased NPY. This study also supports a role for the CB1R and GABA in the mechanisms underlying weight gain side-effects, possibly by altering POMC transmission. Metabolic dysfunction can be modelled in the female rat using low, clinically-comparable olanzapine doses when administered in line with the half-life of the drug.

1.5.4 Summary Conclusion

In conclusion, numerous aspects of clinically-reported metabolic dysfunction side-effects can be modelled in the female Sprague Dawley rat following 14 days' olanzapine treatment (t.i.d.). Olanzapine treatment alters a number of neurotransmission signalling systems in regions of the brain that regulate appetite and metabolism. These changes occur in a manner that can favour positive energy balance and may contribute to the metabolic dysfunction side-effects of olanzapine. Results of the present studies suggest that disruption to M3R signalling may underlie its diabetogenic liability and contribute in part to its weight gain side-effects. Changes to components of melanocortinerbic, GABAergic and cannabinoid neurotransmission in the hypothalamus and brainstem during olanzapine treatment may contribute to the mechanisms underlying its obesogenic liability.

The importance of olanzapine dosage has been illustrated in the present series of studies, with a significant response observed in most parameters following 1.0 or 2.0mg/kg olanzapine (t.i.d.), but inconsistently following 0.5mg/kg olanzapine, while 0.25mg/kg olanzapine was insufficient to induce changes in most aspects examined. These findings demonstrate that when administered in accordance with the half-life of olanzapine in the rat (8-hourly), metabolic dysfunction can be modelled in the female rat using lower dosages that may better reflect the clinical scenario.

CHAPTER TWO

OLANZAPINE TREATMENT AND METABOLIC DYSFUNCTION: A DOSE RESPONSE STUDY IN FEMALE SPRAGUE DAWLEY RATS

Reprinted from Behavioural Brain Research, 217(2), Weston-Green K, Huang XF and Deng C, Olanzapine treatment and metabolic dysfunction: a dose response study in female Sprague Dawley rats, 337-346, Copyright (2011), with permission from Elsevier.



Research report

Olanzapine treatment and metabolic dysfunction: a dose response study in female Sprague Dawley rats

Katrina Weston-Green^{a,b}, Xu-Feng Huang^{a,b}, Chao Deng^{a,b,*}^a Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, Wollongong, 2522, NSW, Australia^b Schizophrenia Research Institute, 384 Victoria Street, Darlinghurst, 2010, NSW, Australia

ARTICLE INFO

Article history:

Received 30 June 2010

Received in revised form 20 October 2010

Accepted 29 October 2010

Available online 4 November 2010

Keywords:

Olanzapine

Weight gain

Animal model

Dose

Metabolic side-effect

Antipsychotic

ABSTRACT

Second generation antipsychotics are commonly prescribed for the treatment of schizophrenia, however some can induce metabolic dysfunction side-effects such as weight gain, obesity and diabetes. Clinical reports suggest olanzapine alters satiety signals, although findings appear conflicting. Previous animal model studies have utilised a range of olanzapine dosages, however the dosage that better mimics the human scenario of olanzapine-induced weight gain is unclear. Female Sprague–Dawley rats were treated orally, three times daily with olanzapine (0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg), self-administered in a sweet cookie dough pellet at eight-hourly intervals or vehicle ($n = 12/\text{group}$) for 14-days. Olanzapine orally self-administered in multiple doses (eight-hourly intervals) may circumvent a drop in plasma drug concentration and ensure the maintenance of a consistently high olanzapine level in the rat. Olanzapine increased body weight (0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg), food intake (2.0 mg/kg) and feeding efficiency (0.5–2.0 mg/kg), with no effect on water intake. Subcutaneous inguinal (1.0 mg/kg, 2.0 mg/kg) and intra-abdominal perirenal fat were increased (2.0 mg/kg), but not interscapula brown adipose tissue. Olanzapine increased circulating ghrelin and cholecystokinin, but had no effect on peptide YY_{(3–36)}}. Olanzapine decreased insulin (0.25–2.0 mg/kg) and locomotor activity in the open field arena (0.5–2.0 mg/kg). A low dosage of 0.25 mg/kg olanzapine had no effect on most parameters measured. Olanzapine-induced weight gain is associated with hyperphagia, enhanced feeding efficiency and adiposity, decreased locomotor activity and altered satiety signaling. The animal model used in the present study of self-administered oral olanzapine treatment (i.d.) at a dosage range of 0.5–2.0 mg/kg (but not 0.25 mg/kg) mimics aspects of the clinic.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Compared to first generation classical antipsychotics, second generation antipsychotic drugs (SGAs) possess enhanced tolerability, with reduced risk of extra-pyramidal side effects, and an efficacy to treat multiple domains of schizophrenia [1–3], but are associated with numerous adverse side-effects including metabolic syndrome. Metabolic side-effects such as weight gain, adiposity, dyslipidaemia, glucose dysregulation and insulin resistance are particularly problematic, leading to further social and medical consequences including obesity, cardiovascular disease, type II diabetes and non-compliance to medication. In addition, the sedentary lifestyle predominant in schizophrenia patients [4–6] may

further exacerbate the on-set of metabolic syndrome. Some potential mechanisms of SGA-induced weight gain have emerged in the past few years, including from our laboratory [7–11,12] and others [13,14]; see reviews by [15,16]), however metabolism is a complex issue and an indisputable hypothesis remains elusive.

Olanzapine is particularly notorious for its weight gain side-effect [2,5], with reports indicating increases in body weight of 4.15 kg within 10-weeks of treatment [17] and 15.5 kg after 1-year [18] in some patients. Weight gain is associated with increased subcutaneous and intra-abdominal fat mass [19], with no effect on lean body mass [20,21]. Evidence suggests that olanzapine may alter appetite/adiposity hormones such as cholecystokinin (CCK), peptide YY (PYY), insulin and ghrelin. For example, chronic olanzapine treatment increases plasma insulin and induces insulin resistance in humans [22,23] and rats [24], even after a single acute dose of olanzapine, risperidone or clozapine [25,26]. Olanzapine may also alter CCK levels, as Vidarsdottir et al. [27] reported a slight preprandial morning elevation in plasma CCK levels in males, but no change in plasma PYY levels under these conditions. However, whether these results can be replicated in a rodent model is unknown. Reports on the effects of olanzapine on ghrelin levels in humans

Abbreviations: CCK, cholecystokinin; PYY, peptide YY; SD rat, Sprague–Dawley rat.

* Corresponding author at: School of Health Sciences, University of Wollongong, Northfields Avenue, Wollongong, 2522, NSW, Australia. Tel.: +61 2 4221 4934; fax: +61 2 4221 8130.

E-mail address: chao@uow.edu.au (C. Deng).

0166-4328/\$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2010.10.039

are inconsistent [13,28–30] (also see [31] for review), which may be attributed experimental variables such as gender, body mass index at the time of testing, olanzapine dosage, length of treatment, concomitant treatments, history of illness and treatment [32], and a biphasic response of ghrelin to antipsychotic treatment duration [33]. In order to further study the effects of olanzapine on these important metabolic hormones there is a need for an appropriate animal model that closely resembles the human scenario of olanzapine-induced weight gain, including adiposity, locomotor activity and appetite signal interference, in the absence of the variables associated with some human studies.

For the past decade scientists have worked to establish an animal model that mimics human antipsychotic-induced weight gain, however inconsistencies between basic research and the clinic are a long-standing debate. Cocurello and Moles [15] provide a recent review of rat and mouse models of SGA-induced metabolic dysfunction, and discussion on findings in the clinic compared to basic science. One of the issues overshadowing animal modeling of SGA-induced metabolic dysfunction is gender sensitivity, with reports of unsuccessful modeling of SGA-induced weight gain in the male rat [34,35,24,36,37]. However, some aspects of metabolic side-effect can be modeled in the male rat as risperidone induces body weight gain and hyperphagia in the male rat [38], acute olanzapine treatment increases food intake in a run-way paradigm [39], acute clozapine and olanzapine treatment, but not haloperidol, increases fat intake [40], and van der Zwaal et al. [101] recently reported increased food intake and meal size, and decreased locomotor activity in male rats after olanzapine treatment. Minet-Ringuet et al. [35] reported enhanced adiposity in male rats following olanzapine treatment, but no weight gain. However shortly after, the same group reported successful weight gain and increased adiposity in the male rat by altering some experimental conditions, i.e. 0.5 mg/kg, 2.0 mg/kg olanzapine dosage self-administered mixed with food [41]. These methods [41] were recently modified by Shobo et al. [42], who also reported success in modeling hyperphagia and enhanced adiposity in the male rat following chronic 5 mg/kg olanzapine mixed with food, and hyperphagia after an acute injection of 2 mg/kg olanzapine. The self-administration method of antipsychotic dosage may better mimic the oral route of drug administration in the clinic [43], and reduce stress response inevitable during daily subcutaneous or intraperitoneal injection and oral gavage [44].

Previous studies have reported olanzapine's ability to increase food intake, weight gain and adiposity in female rats at dosages ranging from 0.5 to 8 mg/kg [24,34,37,45–47], but not at 20 mg/kg/day [24,37]. Thus, the most appropriate dosage that better mimics the multiple aspects of human olanzapine-induced weight gain is unclear. Therefore, we examined the dose-dependent effects of olanzapine on food and water intake, body weight gain, abdominal and subcutaneous adiposity and locomotor activity, as well as circulating metabolic hormones, CCK, PYY_(3–36), insulin and ghrelin in order to assist in understanding the dosage that best mimics aspects of the clinic. Additionally, the present study employed an oral self-administration method for olanzapine delivery, which resembles human drug administration, with a dosage of three times per day to ensure a consistently high concentration of olanzapine in the rat.

2. Experimental procedures

2.1. Animals and diet

Female SD rats (7 weeks old) were obtained from the Animal Resources Centre (Perth, WA, Australia), housed at 22°C, on a 12-h light–dark cycle (lights on: 07:00 h), and allowed *ad libitum* access to water and standard laboratory chow diet (3.9 kcal/g; 10% fat, 74% carbohydrate, 16% protein) throughout the study. Animals were randomly assigned to one of the following treatments: 0.25, 0.5, 1.0 or 2.0 mg olanzapine/kg (Eli Lilly, Indianapolis, IN, USA), or vehicle ($n = 12$), three times daily at

eight-hourly intervals. In the rat, the half-life of olanzapine is 2.5 h in the plasma and 5.1 h in the brain, however levels are still high after 8 h [48]. Therefore, in the present study rats were administered olanzapine three times/day to ensure a consistently high drug concentration to better mirror the human scenario of oral administration once per day. Following 1 week habituation, animals underwent a teaching period to self-administer a sweet cookie dough pellet three-times per day for 1 week, and were sham-weighed daily to minimise handling stress during treatment.

Cookie dough (62% carbohydrate, 22% protein, 6% fibre, 10% vitamins and minerals) administration methods were employed as previously reported by our laboratory [10]. Briefly, a mixture of cornstarch (30.9%), sucrose (30.9%), gelatine (6.3%), casein (15.5%), fibre (6.4%), minerals (8.4%) and vitamins (1.6%) was produced. Olanzapine tablets were separated from their coating, then pulverized using a mortar and pestle. The assigned dosage of powdered olanzapine was added to the measured dry ingredients. Water was added to achieve a dry-dough consistency immediately prior to administration and a 0.3 g cookie-dough pellet (containing the assigned olanzapine dosage) was offered by metal spoon at eight-hourly intervals (three pellets/day) for 14-days. Animals were observed during treatment administration to ensure complete consumption of each pellet, and were weighed daily approximately mid-way through their wake cycle. Food and water intake were recorded daily and results were corrected for spillage. All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

2.2. Behavioural analysis: open field testing

On treatment days 8–10, animals were subjected to open field behavioural testing to examine locomotor activity, as previously described by our group [49]. Briefly, a single animal was placed in the centre of a black square arena (60 cm × 60 cm wide, 40 cm high walls, 100 lx). Animal behaviour was recorded for 30-min and analysed using Ethovision video-tracking software (Noldus Information Technology, Wageningen, The Netherlands). Central and peripheral duration (s) and frequency, as well as total distance travelled (cm), mean velocity (cm/s) and rearing data were recorded.

2.3. Hormonal and post mortem adiposity measurements

Animals were fasted for 4–6 h, then euthanized with sodium pentobarbitone. Upon sedation, blood was removed from the left ventricle for hormonal testing (CCK, PYY_(3–36), insulin and total ghrelin). Samples for each hormone were separately collected in tubes containing 2Na-EDTA, aprotinin, K₂EDTA or heparin, immediately centrifuged, and plasma was aliquoted then stored at –20°C. Fasting circulating insulin levels were measured using commercially available ELISA kits (Linco Research, MO, USA), whilst RIA kits were used to detect CCK, PYY_(3–36) (Phoenix Pharmaceuticals, CA, USA) and total ghrelin (Linco Research, MI, USA) levels.

Inguinal, perirenal and periovary white fat pads, as well as sub-scapula brown fat pads, were dissected and individually weighed.

2.4. Data analysis

Data were statistically analysed using SPSS (version 15, SPSS, Chicago, IL, USA). One-way ANOVAs were used to observe the dosage response of olanzapine on total body weight, food and water intake, adipose tissue, insulin, CCK and PYY_(3–36). Two-way repeated ANOVAs (DOSAGE × DAYS as repeated measures) were employed for cumulative weight gain, food and water intake. Multiple comparisons were performed using post hoc Dunnett-*T* tests. Where Kolmogorov–Smirnov tests revealed abnormal data distribution, Kruskal–Wallis tests were utilised followed by Mann–Whitney *U* post hoc analysis. Correlations were identified using Pearson's correlation tests or Spearman's correlation tests for non-parametric data. Data was considered significant when $p < 0.05$.

3. Results

3.1. Body weight

A one-way ANOVA revealed a significant effect of olanzapine dosage on total body weight gain ($F_{4,55} = 7.25$, $p < 0.01$). Post hoc analysis identified a significant increase in total body weight following 0.5 mg/kg and 1.0 mg/kg ($p < 0.05$) olanzapine/kg compared to controls, with the highest increase (+45%) observed in the 2.0 mg/kg treatment group ($p < 0.01$), but no change in the 0.25 mg/kg group (Table 1). A two-way repeated ANOVA (DOSAGE × DAYS) of cumulative body weight gain revealed significant effects of dosage ($F_{4,55} = 6.40$, $p < 0.01$) and days ($F_{13,715} = 331.78$, $p < 0.01$), and a significant interaction between the two factors ($F_{52,715} = 7.61$, $p < 0.01$). Cumulative weight gain was

Table 1

Mean body weight and fat pad mass (g; mean \pm SEM) in female Sprague–Dawley rats treated with 0.25, 0.5, 1.0 and 2.0 mg/kg olanzapine or control (vehicle) for 14-days (three times/day).

	Control	Olanzapine dosage			
		0.25 mg	0.5 mg	1.0 mg	2.0 mg
<i>Body weight (g)</i>					
IBW	190.7 \pm 3.4	186.0 \pm 3.3	190.0 \pm 2.6	190.1 \pm 3.5	194.9 \pm 4.0
FBW	215.3 \pm 5.8	209.2 \pm 4.8	225.3 \pm 4.3*	227.8 \pm 6.9*	239.4 \pm 6.6**
BWG	24.5 \pm 3.0	23.1 \pm 2.5	35.2 \pm 2.9*	37.7 \pm 4.3*	44.5 \pm 3.8**
<i>Fat pad mass (g)</i>					
Inguinal	3.0 \pm 0.2	2.8 \pm 0.2	3.8 \pm 0.2	4.9 \pm 0.4**	5.2 \pm 0.3**
Perirenal	2.9 \pm 0.3	2.7 \pm 0.2	3.5 \pm 0.3	3.9 \pm 0.4	4.4 \pm 0.3*
Periovary	3.4 \pm 0.3	2.7 \pm 0.3	3.9 \pm 0.3	4.2 \pm 0.4	4.4 \pm 0.3
Subcapula (BAT)	0.4 \pm 0.0	0.4 \pm 0.0	0.4 \pm 0.0	0.5 \pm 0.0	0.5 \pm 0.3
Total white fat	9.3 \pm 0.8	8.3 \pm 0.6	11.3 \pm 0.7	12.9 \pm 1.1**	14.0 \pm 0.9**
Total white fat/FBW (%)	4.3 \pm 0.2	4.0 \pm 0.3	5.0 \pm 0.2	5.6 \pm 0.3**	5.8 \pm 0.3**
<i>Length (mm)</i>					
Body	197.9 \pm 3.0	198.9 \pm 2.5	196.7 \pm 2.4	200.9 \pm 2.2	198.9 \pm 3.0
Tibial	47.1 \pm 0.4	47.1 \pm 0.3	47.2 \pm 0.3	47.3 \pm 0.4	47.2 \pm 0.5

IBW: initial body weight, FBW: final body weight, BWG: body weight gain.

* $p < 0.05$ vs. control.

** $p < 0.01$ vs. control.

significant after days 4–5 in rats treated with 0.5, 1.0 and 2.0 mg/kg olanzapine compared to controls (Fig. 1A).

3.2. Food intake

Olanzapine dosage had a significant effect on total cumulative food intake ($F_{4,55} = 3.09$, $p < 0.05$), with a 15% increase observed in the 2.0 mg/kg olanzapine treatment group ($p < 0.01$) (Fig. 1B). Cumulative food intake data was divided into weeks 1 and 2 and further analysis of week 2 data revealed a significant increase in cumulative food intake in the 2 mg/kg treatment group compared

to controls ($p < 0.01$, days 8–14). Olanzapine increased feeding efficiency (grams of weight gained/grams of food consumed) ($F_{4,55} = 8.03$, $p < 0.01$), in animals treated with 0.5 mg/kg ($p < 0.05$), 1.0 mg/kg and 2.0 mg/kg ($p < 0.01$) olanzapine, but not 0.25 mg/kg ($p > 0.05$), compared to controls (Fig. 1C).

3.3. Water intake

A two-way repeated ANOVA (DOSAGE \times DAYS) of cumulative water intake showed a significant effect of days ($p < 0.01$), but not

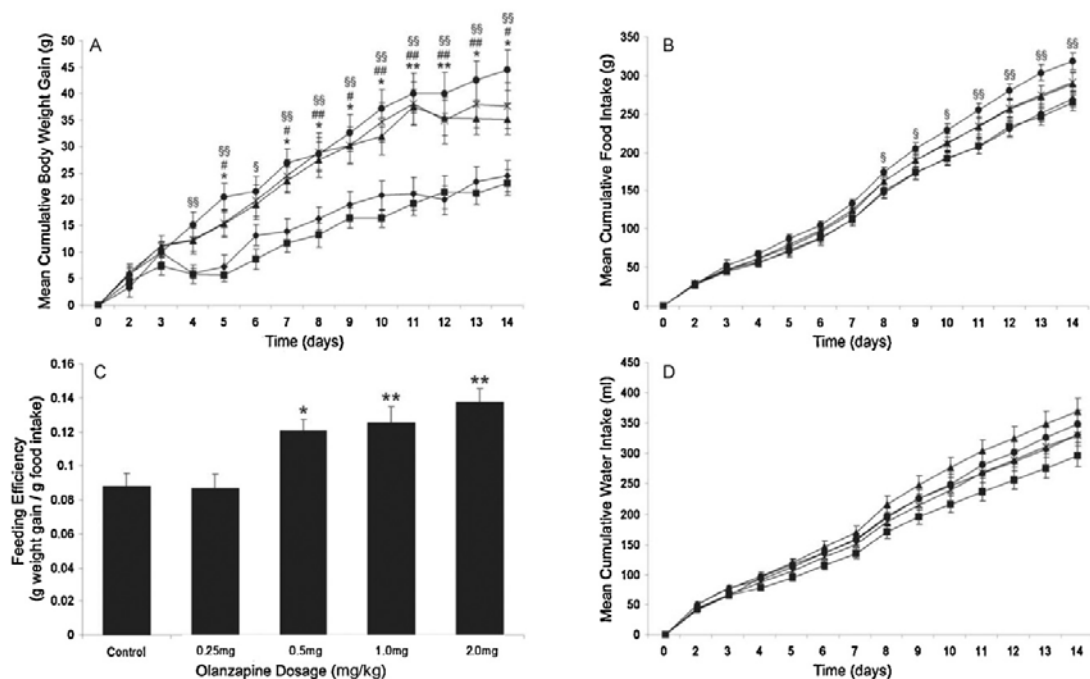


Fig. 1. (A) cumulative body weight gain (\pm SEM); (B) cumulative food intake (\pm SEM); (C) feeding efficiency (gram weight gain/gram food intake \pm SEM); and (D) cumulative water intake (ml \pm SEM) in female Sprague–Dawley rats treated with 0.25, 0.5, 1.0 and 2.0 mg/kg olanzapine or control (vehicle) for 14-days (three times/day). (A, B, and D) * $p < 0.05$, ** $p < 0.01$; 0.5 mg/kg vs. control, # $p < 0.05$, ## $p < 0.01$; 1.0 mg/kg vs. control, \$\$\$ $p < 0.05$, **** $p < 0.01$; 2.0 mg/kg vs. control (■: vehicle/control, ♦: 0.25 mg/kg, ▲: 0.5 mg/kg, ×: 1.0 mg/kg, ●: 2.0 mg/kg olanzapine) (C) * $p < 0.05$, ** $p < 0.01$ vs. control.

Table 2

Fasting appetite hormone levels (fmol/ml \pm SEM) in female Sprague–Dawley rats treated with 0.25, 0.5, 1.0 and 2.0 mg/kg olanzapine or control (vehicle) for 14-days (three times/day).

	Control	Olanzapine dosage			
		0.25 mg	0.5 mg	1.0 mg	2.0 mg
Insulin	170.3 \pm 30.9	71.9 \pm 8.9**	65.9 \pm 8.0**	73.9 \pm 4.1**	84.7 \pm 11.0**
Total ghrelin	149.0 \pm 10.5	378.9 \pm 74.1*	453.0 \pm 52.0**	407.0 \pm 78.2*	422.6 \pm 74.4*
Cholecystokinin	134.8 \pm 15.5	188.4 \pm 15.1	224.5 \pm 21.6**	165.6 \pm 15.9	203.6 \pm 17.0**
Peptide YY _(3–36)	55.7 \pm 9.9	61.6 \pm 8.9	49.6 \pm 7.8	46.2 \pm 5.3	52.6 \pm 9.3

* $p < 0.05$ vs. control.

** $p < 0.01$ vs. control.

dosage ($F_{4,54} = 2.13$, $p = 0.09$), and a significant interaction between the two factors ($F_{52,702} = 2.58$, $p < 0.01$) (Fig. 1D).

3.4. Adiposity

The effect of olanzapine dosage of adiposity is shown in Table 1. There was a significant effect of olanzapine dosage on subcutaneous inguinal ($F_{4,55} = 16.11$, $p < 0.01$), intra-abdominal perirenal ($F_{4,55} = 4.94$, $p < 0.01$) and periovary ($F_{4,55} = 3.47$, $p < 0.01$) white fat mass. Inguinal fat mass increased following 1.0 and 2.0 mg/kg olanzapine treatment ($p < 0.01$), with a trend towards significance in the 0.5 mg/kg olanzapine group ($p = 0.07$) compared to controls. Perirenal fat mass increased in the 2.0 mg/kg treatment group ($p < 0.01$) with a trend towards significance in the 1.0 mg/kg treatment group ($p = 0.06$) compared to controls. A trend was also observed ($p = 0.07$) in the 2.0 mg/kg olanzapine treatment group in relation to periovary fat mass. However, olanzapine had no effect on subscapula brown fat mass compared to controls ($p = 0.33$). Olanzapine increased total white fat mass (inguinal + perirenal + periovary) ($F_{4,55} = 8.35$, $p < 0.01$) in the 1.0 mg/kg (+28%) and 2.0 mg/kg (+33%) olanzapine treatment groups compared to controls (both $p < 0.01$). There was no effect of olanzapine treatment on post mortem body and tibial length ($p = 0.84$ and 0.99 , respectively), which were measured as internal controls (Table 1).

3.5. Hormones

The effect of olanzapine dosage on plasma hormones is shown in Table 2. A one-way ANOVA revealed a significant effect of olanzapine dosage on fasting plasma insulin levels ($F_{4,51} = 7.20$, $p < 0.01$), with a significant decrease ($p < 0.01$) in insulin levels observed in all dosage groups compared to controls. Olanzapine dosage significantly increased total ghrelin following 0.25 mg/kg, 1.0 mg/kg and 2.0 mg/kg olanzapine treatment ($p < 0.05$) and 0.5 mg/kg olanzapine treatment ($p < 0.01$) compared to controls. Fasting plasma CCK levels increased ($F_{4,49} = 4.21$, $p < 0.01$) following 0.5 mg/kg and 2.0 mg/kg olanzapine ($p < 0.01$), whilst the 0.25 mg/kg treatment group showed a trend towards significance ($p = 0.07$), but not in animals treated with 1.0 mg/kg olanzapine compared to

controls ($p = 0.27$). Fasting plasma PYY_(3–36) remained unchanged ($p = 0.56$).

3.6. Behavioural: open field testing

Behavioural testing results are shown in Table 3. Total distance travelled significantly decreased with increasing olanzapine dosage ($F_{4,55} = 14.38$, $p < 0.01$) in animals treated with 0.5 mg/kg (–29%), 1.0 mg/kg (–40%) and 2.0 mg/kg (–60%) olanzapine compared to controls, but not 0.25 mg/kg ($p = 0.39$). Mean velocity of travel also decreased with increasing olanzapine dosage ($F_{4,55} = 140.44$, $p < 0.01$) in the 0.5 mg/kg (–29%), 1.0 mg/kg (–46%) and 2.0 mg/kg (–53%) olanzapine treatment groups compared to controls ($p < 0.01$) ($p = 0.41$ for 0.25 mg/kg). Animals in the 0.5 mg/kg ($p < 0.05$), 1.0 mg/kg and 2.0 mg/kg ($p < 0.01$) olanzapine dosage groups, but not the 0.25 mg/kg group ($p = 0.84$), displayed significantly lower rearing frequency than controls.

Centre frequency (number of entries from periphery to centre) and duration (both $p < 0.01$) decreased in the 1.0 mg/kg and 2.0 mg/kg treatment groups ($p < 0.01$). Olanzapine decreased peripheral frequency and increased peripheral duration in the 1.0 mg/kg and 2.0 mg/kg olanzapine treatment groups ($p < 0.01$), but not in the 0.25 mg/kg or 0.5 mg/kg groups ($p > 0.05$), compared to controls.

3.7. Correlations

There was a significant positive correlation between mean total body weight gain and total food intake ($r = 0.84$, $p < 0.01$) (Fig. 2A) and total white fat mass ($r = 0.76$, $p < 0.01$) (Fig. 2B). In addition, feeding efficiency was significantly correlated to total white fat mass ($r = 0.68$, $p < 0.01$, Fig. 2C). There was no significant correlation between insulin and total body weight gain or total food intake. A significant positive correlation was observed between total ghrelin and total body weight gain ($r = 0.44$, $p < 0.01$), total food intake ($r = 0.35$, $p < 0.05$), and total white fat mass ($r = 0.43$, $p < 0.01$), whilst CCK showed a tendency towards a significant relationship with total white fat mass ($r = 0.25$, $p = 0.07$). In terms of behavioural analysis, total distance travelled negatively correlated to total body

Table 3

Open field testing in female Sprague–Dawley rats treated with 0.25, 0.5, 1.0 and 2.0 mg/kg olanzapine or control (vehicle) for 14-days (three times/day). Data is expressed as mean \pm SEM.

Behaviour	Control	Olanzapine dosage			
		0.25 mg	0.5 mg	1.0 mg	2.0 mg
Open field test					
Total distance travelled (cm)	8497.9 ± 676.4	7736.4 ± 845.5	6045.6 ± 357.0*	4612.3 ± 360.8**	3428.0 ± 339.7**
Mean velocity (cm/s)	4.7 ± 0.4	4.3 ± 0.5	3.4 ± 0.2**	2.6 ± 0.2**	2.2 ± 0.3**
Rearing frequency	25.1 ± 4.4	26.7 ± 4.9	12.3 ± 1.4*	10.9 ± 5.5**	5.9 ± 1.6**
Centre duration (s)	9.7 ± 2.9	10.6 ± 4.1	6.1 ± 2.0	2.2 ± 0.8**	1.5 ± 0.5**
Periphery duration (s)	1790.3 ± 2.9	1789.5 ± 4.1	1793.9 ± 2.0	1797.8 ± 0.8**	1798.5 ± 0.5**
Centre frequency	12.5 ± 2.3	11.3 ± 3.1	7.25 ± 1.4	2.7 ± 0.6**	2.3 ± 0.6**
Periphery frequency	13.5 ± 2.3	12.3 ± 3.1	8.3 ± 1.4	3.7 ± 0.6**	3.3 ± 0.6**

* $p < 0.05$ vs. control.

** $p < 0.01$ vs. control.

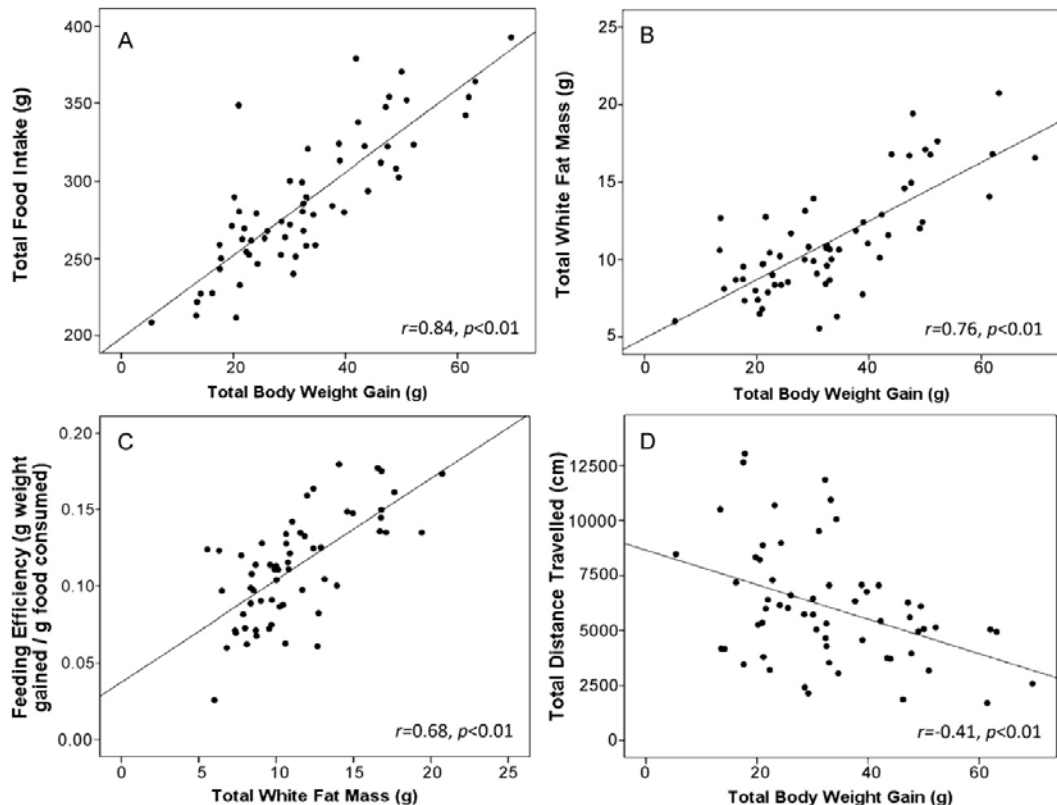


Fig. 2. Correlations between total body weight gain (g) and (A) total food intake (g), (B) total white fat mass (g), (D) total distance travelled (cm/30 min) in an open field testing arena, (C) correlation between feeding efficiency (gram weight gained/gram food consumed) and total white fat mass (g), in female Sprague–Dawley rats orally self-administered olanzapine (three times/day).

weight gain ($r = -0.41$, $p < 0.01$, Fig. 2D) and total white fat mass ($r = -0.50$, $p < 0.01$). Velocity negatively correlated to total body weight gain ($r = -0.35$, $p < 0.01$) and total white fat mass ($r = -0.48$, $p < 0.01$).

4. Discussion

The present study investigated the dosage response (range 0.25 mg/kg–2.0 mg/kg, three times/day) of body weight, food and water intake, appetite hormones ghrelin, insulin, CCK and PYY_(3–36), and locomotor behaviour to olanzapine treatment in free-feeding female SD rats. To our knowledge this is the first report of olanzapine's effect on CCK and PYY_(3–36) in the rat model. Olanzapine-induced weight gain was associated with altered satiety signaling, hyperphagia, enhanced subcutaneous and intra-abdominal fat, and reduced locomotor activity. Olanzapine increased plasma CCK and ghrelin, and decreased insulin, whilst no change was observed in plasma PYY_(3–36). The animal model used in the present study of self-administered oral olanzapine treatment (t.i.d.) at a dosage range of 0.5–2.0 mg/kg (but not 0.25 mg/kg) mimics aspects of the clinic. This study confirms that olanzapine can induce major metabolic disturbances during the first phase of repeated administration.

4.1. Body weight gain, food and water intake

Olanzapine significantly increased body weight gain in the 0.5, 1.0 and 2.0 mg/kg treatment groups after 4–5-days in the

present study. Our findings coincide with previous studies that have described the effect of olanzapine on weight gain in the female rat [10,24,34,37,45–47,50,51], and in mice orally self-administered SGAs [52,53]. In humans, weight gain is most problematic after 6–8 weeks olanzapine treatment, with the highest rate of gain during this period, plateauing after 1-year (reviewed in [54]). Coinciding with the clinic, a study from our laboratory reported that chronic olanzapine treatment (0.5 mg/kg) induced significant body weight gain in female rats in the first 8-weeks of treatment, with the largest change in body weight observed during the first week of treatment [10]. Therefore, the model used in the present study was based on 14-days olanzapine administration, as maximal weight gain can be observed during this treatment period in the female rat. In the present study, we observed a dosage response of olanzapine to weight gain, with a higher dosage of 2.0 mg/kg inducing a greater effect. Coinciding with our findings, Nemeroff [55] reported an olanzapine dosage response to weight gain side-effect in schizophrenia patients, whereby increased dosage (range 1–17.5 mg/day) was associated with enhanced weight gain.

Olanzapine increased total cumulative food intake following 2.0 mg/kg treatment, and enhanced feeding efficiency in the 0.5, 1.0 and 2.0 mg/kg treatment groups. Coinciding with our results, previous studies have reported a significant increase in caloric intake in olanzapine-treated patients [20,56] and female rats [11,24,37,45,51]. In addition, we found that food intake was positively correlated to body weight gain, suggesting weight gain may be due to enhanced energy consumption. Interestingly, food intake was not significantly increased in the 0.5 and 1.0 mg/kg treatment

groups in this study, despite a significant increase in body weight in these animals. Even in the 2.0 mg/kg treatment group, significant hyperphagia was apparent after 8-days treatment, even though weight gain was significant after only 4-days treatment. This result may be partially attributed to the decreased locomotor activity observed in the present study (discussed in Section 4.4), decreased energy expenditure, as reported in other studies following olanzapine treatment in rats and mice [57,58], or other factors including olanzapine's ability to directly influence adipogenesis [14].

In the present study there was no effect of olanzapine on water intake. This result reflects the previous findings from our laboratory and similar animal model studies [10,46,57,59], however one group reported a significant decrease in water intake following 5 mg and 20 mg/kg/day olanzapine treatment in rats [37]. In humans, 10–20% of schizophrenia patients suffer polydipsia [60], however unlike clozapine [61], olanzapine lacks therapeutic effect on polydipsia [62], and olanzapine's effect on fluid intake in the general schizophrenia population is unclear. Olanzapine can cause dry-mouth in some patients due to its anti-cholinergic properties, which may influence fluid consumption.

4.2. Adiposity

A dosage-response of olanzapine to adiposity was observed in the present study. Olanzapine enhanced white fat mass by 28–33% in the two higher dosage groups, specifically inguinal (1 mg and 2 mg/kg), perirenal (2 mg/kg), and a slight increase in periovary adipose tissue. Our findings confirm previous reports that olanzapine increases intra-abdominal fat mass in rats following 4 mg olanzapine treatment [46], and perirenal fat mass following 2 mg and 4 mg, but not 8 mg, treatment [47]. Chintoh et al. [63] reported increased visceral fat in rats treated with 7.5 mg/kg/day olanzapine, but not at a lower dosage of 2 mg/kg/day. Olanzapine-induced weight gain is associated with an increase in body fat mass in humans [20], however the precise localisation of olanzapine-induced fat accumulation in humans is unclear. Some studies have reported olanzapine-induced increases in waist circumference, suggestive of truncal body fat deposition [21,22]. Ryan et al. [100] found a significant increase in intra-abdominal and subcutaneous fat in first episode schizophrenia patients following 6-months olanzapine treatment, and similar findings were reported by Zhang et al. [19] following treatment with risperidone and quetiapine. The relationship between increased abdominal adiposity, particularly visceral adipose deposits, and risk of diabetes and cardiovascular disease is well-documented. Therefore, in the present study we focused on perirenal and gonadal periovary adipose mass, as these are major deposits within the abdominal cavity, as well as the inguinal fat pad, a subcutaneous adipose deposit in the rat.

The strong positive relationship between total white fat and body weight gain in the present study suggests that olanzapine-induced weight gain is largely attributed to increased white fat deposition. Evidence shows that olanzapine acts directly in the periphery to increase fat reserves. For example, olanzapine induces adipogenesis *in vitro* through enhanced expression of sterol regulatory element-binding protein 1 (SREBP-1), which together with its related genes promotes the production of adipocytes [14]. Olanzapine also enhances differentiation of pre-adipocytes to mature adipocytes and triglyceride accumulation *in vitro* [14].

Olanzapine had no effect on brown adipose tissue (BAT) mass in the present study. Failure of BAT thermogenesis to adequately compensate weight gain with increased metabolism suggests an olanzapine-induced dysregulation of thermogenesis in the present study, however further investigation is required. Minet-Ringuet et al. [35] reported a slight though significant increase in interscapula BAT in male rats treated with olanzapine for 8-weeks, but no overall weight gain. Increased BAT may have limited the weight

gain in those animals, although BAT mitochondrial thermogenic activity did not differ to controls [35]. However, Stefanidis et al. [57] found olanzapine decreased the usually elevated dark-phase BAT temperature and significantly reduced the expression of the mitochondrial uncoupling protein, UCP1, in the interscapula BAT of female rats [57].

4.3. Hormones

In the present study we reported that 14-days treatment with olanzapine significantly decreased basal insulin in all dosage groups, which was interesting as insulin is commonly classified as an adiposity hormone and circulating levels are proportional to fat mass [64]. Coinciding with our findings, Chiu et al. [65] reported decreased insulin secretion in response to glucose loading in patients treated with olanzapine for 14-days, and Chintoh et al. [66] found that pre-treatment with a single dose of olanzapine significantly decreased insulin levels accompanied by hyperglycaemia during a glucose challenge in male rats. On the other hand, numerous reports indicate that chronic olanzapine treatment increases insulin levels and insulin resistance in humans [22,23,67], and diabetes is a well-documented risk associated with olanzapine treatment. This apparent conflict in results may be reconciled by the hypothesis of a time-dependent response of insulin to olanzapine treatment. A recent study by Chiu et al. [68] reported decreased serum insulin levels in response to a glucose challenge in schizophrenia patients treated with olanzapine for 2-weeks, followed by a return to base-line insulin levels by the fourth week, and increased serum insulin levels by the eighth week of olanzapine treatment. It is possible that the hypoinsulinemia induced by short-term olanzapine treatment may lead to insulin hypersensitivity, which may result in compensatory hyperinsulinemia and insulin-resistance following chronic exposure to olanzapine. A possible mechanism for this finding may be related to the drug binding profile, as olanzapine and clozapine are potent cholinergic muscarinic M3 receptor antagonists [69] and can directly decrease insulin secretion from pancreatic β cells *in vitro* through muscarinic M3 receptor blockade, even in the presence of glucose [70]. Therefore, the decrease in insulin observed in the present study may be representative of olanzapine's direct effect on the pancreas. Muscarinic M3 receptors are also expressed in the dorsal vagal complex of the brainstem (DVC) where they can stimulate beta cell proliferation and insulin secretion [71,72]; therefore central effects of olanzapine on M3 receptors may also be important.

In the present study, olanzapine significantly increased total ghrelin in all treatment groups and ghrelin positively correlated to body weight gain, food intake and white fat mass. An olanzapine-induced increase in ghrelin coincides with the hyperphagia and elevated risk of weight gain side-effect of this drug in the clinic, and suggests a drug-induced dysfunction of central feedback mechanisms or over-secretion of the hormone. The increase in ghrelin observed in the present study is in agreement with previous reports in humans and rats following treatment with olanzapine, clozapine and risperidone [30,73,74]. However, the reported effects of atypical antipsychotic drugs on circulating ghrelin levels in humans are varied, and some studies do show a decrease or no change in ghrelin in patients following olanzapine treatment (reviewed in 31; see also 75–77). The current study may assist to clarify whether results observed in human studies reflect an olanzapine-induced effect on ghrelin, or are due to confounds such as concomitant treatments (e.g. benzodiazepines, which have their own metabolic impact [78]), treatment history and history of illness [32]. In addition, a biphasic response of ghrelin to antipsychotic treatment may also offer reconciliation to reported confounds, as Sentissi et al. [33] observed that ghrelin levels tended to be suppressed during early treatment and elevated at later stages. In the present study,

we examined the effect of olanzapine on plasma total ghrelin. Biologically active ghrelin involves modification of the peptide by the addition of an acyl group to the third amino acid residue (serine). In humans and rodents, *n*-octanoyl is the main acyl-chain that binds to serine, however other acyl-forms of ghrelin have been identified, including the recently discovered *n*-decanoyl [79], which may not be detected by currently available commercial kits.

We observed an increase in CCK levels following 0.5 mg and 2.0 mg/kg treatment, but unexplainably not in the 1.0 mg/kg treatment group. A recent human study found a slight preprandial elevation in plasma CCK levels at breakfast in olanzapine-treated male patients [27], however to our knowledge the present study is the first report of the effect of olanzapine on CCK in an animal model. As CCK is an anorectic hormone that signals the body's satiated state to the brain to decrease food intake [80], the elevated CCK levels observed in the present study following olanzapine treatment may be a compensatory mechanism against further hyperphagia and associated body weight gain, albeit ineffective in the high dosage group (2.0 mg/kg, t.i.d.) of the present study, which showed significant hyperphagia by the second week of olanzapine treatment. However, the inability of elevated CCK levels to effectively suppress feeding in olanzapine-treated animals is not entirely surprising. Firstly, Shrestha et al. [81] found that CCK increases ghrelin secretion from isolated rat stomachs by 200%. Secondly, a study by Burdya et al. [82] found that CCK decreased cannabinoid CB1 receptor (CB1R) expression on vagal afferent neurons. As activation of CB1R induces food intake, Burdya et al. [82] proposed a mechanism by which CCK decreases food intake through inhibition of CB1R expression. We have previously reported that olanzapine decreases CB1R binding density in the dorsal vagal complex of the brainstem [7] and more recently in the hypothalamus (unpublished), brain regions important in the control of food intake. Therefore, it is possible that olanzapine induces CCK dysregulation of satiety inhibition by decreasing CB1R density. An alternative explanation for increased plasma CCK levels may be related to the therapeutic mechanisms by which olanzapine can treat schizophrenia, as decreased CCK may play a role in the disease pathophysiology. For example, schizophrenia patients have low cerebrospinal fluid levels of CCK [83] and decreased CCK mRNA expression in the temporal, frontal and entorhinal cortices [84,85].

Olanzapine treatment had no effect on the level of circulating PYY_(3–36), which coincides with findings in male patients [27]. These results suggest that plasma PYY may not be involved in olanzapine-induced weight gain, even though fasting and post-prandial plasma PYY levels are reduced in human obesity [86,87], and peripheral PYY administration reduces food intake in lean and obese humans [86]. A study from our laboratory reported alterations in PYY receptor binding density in various regions of the rat brain following chronic (36-days) olanzapine treatment [88] indicating olanzapine does have some effect on PYY signaling pathways.

4.4. Behavioural testing

Olanzapine decreased locomotor activity and exploratory behaviour during open field testing in a dose-dependent manner that exhibited a trend of diminished activity with increased dosage. Olanzapine may have induced a sedative effect, particularly in the high dosage group (2.0 mg/kg, three times/day), which may exacerbate weight gain in these animals due to decreased energy expenditure. Albaugh et al. [24] reported sedation in animals receiving 20 mg/kg/day olanzapine, which resulted in hypophagia, but not in the lower dosages of 4, 8 and 12 mg/kg/day. In the present study, hyperphagia was only observed in the high dosage 2 mg/kg (three times/day) olanzapine group, therefore although it should

still be considered, it is unlikely that sedation would have interfered with food intake. Clinical studies have reported an increased incidence of sedation with increased olanzapine dosage from 2.5 to 17.5 mg [89].

There was a negative relationship between locomotor activity and body weight gain, and fat mass in the present study indicating that the metabolic dysfunction induced by olanzapine treatment is at least partially due to decreased active energy expenditure. Resting energy expenditure was not measured in the present study. Some clinical studies have shown that olanzapine has no effect on resting energy expenditure [21,56], whilst others have reported decreased energy expenditure following olanzapine and clozapine treatment in the clinic and in mice [58,90–92]. Additionally, 24-h home cage activity may be a more suitable paradigm to measure general activity in the living environment. Consistent with our findings, a decrease in locomotor activity has been reported in similar animal model studies following treatment with olanzapine [45,63], haloperidol and risperidone [93,94].

4.5. The animal model

The dosages selected for the present study coincide with previous reports of successful modeling of olanzapine-induced weight gain in the female rat using a dosage range of 0.5–8 mg/kg olanzapine [24,34,37,45–47]. In addition, we believe the dosages selected for the present study of 0.5, 1.0 and 2.0 mg/kg olanzapine (t.i.d.) may resemble the clinic, where the recommended clinical dosage range is 5–20 mg/kg, when translated between species based on differences in body surface area between rats and humans [95]. The low dosage of 0.25 mg/kg was included to test the minimum threshold required to induce metabolic dysfunction.

Previous animal model studies of olanzapine-induced weight gain have identified some key considerations that question the validity of this model in its ability to mimic the human scenario. Importantly, studies have shown that clozapine, the antipsychotic with the highest risk for weight gain side-effect in the clinic [17], does not induce weight changes in the rodent model (0.5–24 mg/kg/day clozapine) [34,96]. However, other studies have shown that aspects of clozapine-induced human metabolic dysfunction can be replicated in the rodent, including enhanced adiposity, hypolepinaemia, dyslipidaemia, hyperglycaemia and reduced insulin sensitivity in female rats [96,97]. In male rats, clozapine induces a diabetes-like phenotype through enhanced glucagon secretion, resulting in increased blood glucose and insulin levels [98].

Pertaining to the issue of gender, growing evidence is now surfacing to show gender-related differences in the clinic, where females are more sensitive than males in terms of antipsychotic therapeutic efficacy and weight gain side-effects [99]. In addition, aspects of metabolic dysfunction can be modelled in male rats under certain conditions. Minet-Ringuet et al. [41] successfully modeled olanzapine-induced weight gain in the male rat, supported by a recent study by Shobo et al. [42] who reported hyperphagia and increased adiposity in male rats treated with olanzapine, but not after ziprasidone treatment, when administered via food source (method described by [41]). This suggests that the rodent model may not be as far from the human scenario as previously believed. These studies also illustrate the importance of drug administration method in animal modelling of SGA-induced weight gain. Oral self-administration may be closer to the clinic than other administration methods such as daily subcutaneous or intraperitoneal injection, osmotic minipump, or oral gavage, which may enhance stress response [44]. To our knowledge, the three times daily, oral self-administration procedure of olanzapine or other antipsychotics has not been conducted in male rats, which is a possible factor that may account for their reduced sensitiv-

ity to SGA-induced weight gain, and may be promising for future studies.

In terms of the overall relevance of an animal model of antipsychotic-induced weight gain, olanzapine can induce weight gain and metabolic disturbance in the female and male rat that does mimic aspects of the human scenario. The animal model can be used as a basis for mechanistic studies and provide a framework for potential therapeutics. The new study of olanzapine-induced weight gain in the male rat [42] evidences the importance of more studies to add to the current literature in this expanding field. Our study is the first to report the effects of olanzapine on CCK, PYY_(3–36), and adds to a new concept in insulin response, also illustrating that this field is not exhausted.

5. Conclusion

In the present study olanzapine treatment caused body weight gain, which was associated with an increase in food intake (but not water intake) and feeding efficiency, as well as enhanced subcutaneous and intra-abdominal white fat, coupled with decreased gross motor activity. In addition, olanzapine increased plasma ghrelin and CCK and decreased insulin, but did not influence PYY_(3–36) levels. These results indicate that multiple hormonal systems are involved in olanzapine-induced weight gain. Furthermore, this study illustrated a dose-dependent effect of olanzapine on most of the parameters measured, with a trend showing a greater outcome with increased dosage, and a maximal response following a dosage of 2.0 mg/kg, however most metabolic side-effect parameters measured could be observed after only 0.5 mg/kg. A low dosage of 0.25 mg/kg olanzapine had no effect on body weight even though this dose induced some changes in circulating appetite hormone levels, suggesting that 0.25 mg/kg olanzapine does not reach the threshold dosage for inducing weight gain. A limitation of the present study is possible interference of sedation with metabolic data. Also, successful modeling of antipsychotic-induced metabolic dysfunction using other weight-labile SGAs would further validate this animal model in its ability to mimic the clinic. The animal model used in the present study mimics numerous aspects of the clinical scenario of olanzapine-induced weight gain, however this model is not without limitations, particularly relating to the reduced response of male rats. Conversely, the sensitivity of female rodents to the side-effect of weight gain following olanzapine treatment does appear to mirror reports on the human response.

Conflict of interests

C. Deng has received honorarium from Eli Lilly Australia for presenting at the Cutting Edge Debate Melbourne (2010). The authors have no other relevant affiliations or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Acknowledgements

We thank Ms G. Rahardjo, Ms B. Merryweather, Dr M. Han and Dr Y.H. Yu, who kindly provided laboratory assistance. This study was supported by a University of Wollongong URC grant to C. Deng, and by the Schizophrenia Research Institute, Australia, utilising infrastructure funding from NSW Health.

References

- [1] Keefe RSE, Young CA, Rock SL, Purdon SE, Gold JM, Breier A. One-year double-blind study of the neurocognitive efficacy of olanzapine, risperidone, and haloperidol in schizophrenia. *Schizophrenia Research* 2006;81:1–15.
- [2] Haro JM, Novick D, Suarez D, Roca M. Antipsychotic treatment discontinuation in previously untreated patients with schizophrenia: 36-month results from the SOHO study. *Journal of Psychiatric Research* 2009;43:265–73.

- [3] Kane J, Honigfeld G, Singer J, Meltzer H. Clozapine for the treatment-resistant schizophrenic: a double-blind comparison with chlorpromazine. *Archives of General Psychiatry* 1988;45:789–96.
- [4] Brown S, Birtwistle J, Roe L, Thompson C. The unhealthy lifestyle of people with schizophrenia. *Psychological Medicine* 1999;29:697–701.
- [5] Allison DB, Newcomer JW, Dunn AL, Blumenthal JA, Fabricatore AN, Daumit GL, et al. Obesity among those with mental disorders: a national institute of mental health meeting report. *American Journal of Preventive Medicine* 2009;36:341–50.
- [6] Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, et al. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *The New England Journal of Medicine* 2005;353:1209–23.
- [7] Weston-Green K, Huang X-F, Han M, Deng C. The effects of antipsychotics on the density of cannabinoid receptors in the dorsal vagal complex of rats: implications for olanzapine-induced weight gain. *The International Journal of Neuropsychopharmacology* 2008;11:827–35.
- [8] Deng C, Weston-Green K, Huang X-F. The role of histaminergic H1 and H3 receptors in food intake: a mechanism for atypical antipsychotic-induced weight gain? *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2010;34:1–4.
- [9] Deng C, Weston-Green KL, Han M, Huang X-F. Olanzapine treatment decreases the density of muscarinic M2 receptors in the dorsal vagal complex of rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2007;31:915–20.
- [10] Han M, Deng C, Burne THJ, Newell KA, Huang X-F. Short- and long-term effects of antipsychotic drug treatment on weight gain and H1 receptor expression. *Psychoneuroendocrinology* 2008;33:569–80.
- [11] Huang X-F, Han M, Huang X, Zavitsanos K, Deng C. Olanzapine differentially affects 5-HT2A and 2C receptor mRNA expression in the rat brain. *Behavioural Brain Research* 2006;171:355–62.
- [12] Huang XF, Deng C, Zavitsanos K. Neuropeptide Y mRNA expression levels following chronic olanzapine, clozapine and haloperidol administration in rats. *Neuropeptides* 2006;40:213–9.
- [13] Kluge M, Schuld A, Schacht A, Himmerich H, Dalal MA, Wehmeier PM, et al. Effects of clozapine and olanzapine on cytokine systems are closely linked to weight gain and drug-induced fever. *Psychoneuroendocrinology* 2009;34:118–28.
- [14] Yang L-H, Chen T-M, Yu S-T, Chen Y-H. Olanzapine induces SREBP-1-related adipogenesis in 3T3-L1 cells. *Pharmacological Research* 2007;56:202–8.
- [15] Coccorello R, Moles A. Potential mechanisms of atypical antipsychotic-induced metabolic derangement: clues for understanding obesity and novel drug design. *Pharmacology & Therapeutics* 2010;127:210–51.
- [16] Reynolds GP, Kirk SL. Metabolic side effects of antipsychotic drug treatment—pharmacological mechanisms. *Pharmacology & Therapeutics* 2010;125:169–79.
- [17] Allison D, Mentore J, Heo M, Chandler L, Cappelleri J, Infante M, et al. Antipsychotic-induced weight gain: a comprehensive research synthesis. *The American Journal of Psychiatry* 1999;156:1686–96.
- [18] Zipursky RB, Gu H, Green AL, Perkins DO, Tohen MF, McEvoy JP, et al. Course and predictors of weight gain in people with first-episode psychosis treated with olanzapine or haloperidol. *British Journal of Psychiatry* 2005;187:537–43.
- [19] Zhang Z-J, Yao Z-J, Liu WEN, Fang QUN, Reynolds GP. Effects of antipsychotics on fat deposition and changes in leptin and insulin levels: magnetic resonance imaging study of previously untreated people with schizophrenia. *British Journal of Psychiatry* 2004;184:58–62.
- [20] Eder U, Mangweth B, Ebenbichler C, Weiss E, Hofer A, Hummer M, et al. Association of olanzapine-induced weight gain with an increase in body fat. *The American Journal of Psychiatry* 2001;158:1719.
- [21] Graham KA, Perkins DO, Edwards LJ, Barrier Jr RC, et al. Effect of olanzapine on body composition and energy expenditure in adults with first-episode psychosis. *The American Journal of Psychiatry* 2005;162:118.
- [22] Oriot P, Feys J, Mertens de Wilmars S, Misson S, Ayache A, Fagnart O, et al. Insulin sensitivity, adjusted beta-cell function and adiponectinaemia among lean drug-naïve schizophrenic patients treated with atypical antipsychotic drugs: a nine-month prospective study. *Diabetes & Metabolism* 2008;34:490–6.
- [23] Perez-Iglesias R, Vazquez-Barquero JL, Amado JA, Berja A, Garcia-Unzueta MT, Pelayo-Teran JM, et al. Effect of antipsychotics on peptides involved in energy balance in drug-naïve psychotic patients after 1 year of treatment. *Journal of Clinical Psychopharmacology* 2008;28:289–95.
- [24] Albaugh V, Henry C, Bello N, Hajnal A, Lynch S, Halle B, et al. Hormonal and metabolic effects of olanzapine and clozapine related to body weight in rodents. *Obesity* 2006;14:36–50.
- [25] Houseknecht KL, Robertson AS, Zavadowski W, Gibbs EM, Johnson DE, Rolfe H. Acute effects of atypical antipsychotics on whole-body insulin resistance in rats: implications for adverse metabolic effects. *Neuropsychopharmacology* 2006;32:289–97.
- [26] Boyda HN, Tse L, Procyshyn RM, Wong D, Wu TKY, Pang CC, et al. A parametric study of the acute effects of antipsychotic drugs on glucose sensitivity in an animal model. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2010;34:945–54.
- [27] Vidarsdottir S, Roelfsema F, Streefland T, Holst JJ, Rehfeld JF, Pijl H. Short-term treatment with olanzapine does not modulate gut hormone secretion: olanzapine disintegrating versus standard tablets. *European Journal of Endocrinology* 2010;162:75–83.

- [28] Tanaka K, Morinobu S, Ichimura M, Asakawa A, Inui A, Hosoda H, et al. Decreased levels of ghrelin, cortisol, and fasting blood sugar, but not n-octanoylated ghrelin, in Japanese schizophrenic inpatients treated with olanzapine. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2008;32:1527–32.
- [29] Atmaca M, Tezcan E, Ustundag B. Plasma nitric oxide and leptin values in patients with olanzapine-induced weight gain. *Journal of Psychiatric Research* 2007;41:74–9.
- [30] Esen-Danaci A, Sarandöl A, Taneli F, Yurtsever F, Özlen N. Effects of second generation antipsychotics on leptin and ghrelin. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2008;32:1434–8.
- [31] Jin H, Meyer JM, Mudaliar S, Jeste DV. Impact of atypical antipsychotic therapy on leptin, ghrelin, and adiponectin. *Schizophrenia Research* 2008;100:70–85.
- [32] Gebhardt S, Haberhausen M, Heinzel-Gutenbrunner M, Gebhardt N, Remschmidt H, Krieg J-C, et al. Antipsychotic-induced body weight gain: predictors and a systematic categorization of the long-term weight course. *Journal of Psychiatric Research* 2009;43:620–6.
- [33] Sentissi O, Epelbaum J, Olie J-P, Poirier M-F. Leptin and ghrelin levels in patients with schizophrenia during different antipsychotics treatment: a review. *Schizophrenia Bulletin* 2008;34:1189–99.
- [34] Choi S, DiSilvio B, Unangst J, Fernstrom JD. Effect of chronic infusion of olanzapine and clozapine on food intake and body weight gain in male and female rats. *Life Sciences* 2007;81:1024–30.
- [35] Minet-Ringuet J, Even PC, Goubern M, Tomé D, de Beaupaire R. Long term treatment with olanzapine mixed with the food in male rats induces body fat deposition with no increase in body weight and no thermogenic alteration. *Appetite* 2006;46:254–62.
- [36] Minet-Ringuet J, Even PC, Guesdon B, Tomé D, de Beaupaire R. Effects of chronic neuroleptic treatments on nutrient selection, body weight, and body composition in the male rat under dietary self-selection. *Behavioural Brain Research* 2005;163:204–11.
- [37] Pouzet B, Mow T, Kreilgård M, Velschow S. Chronic treatment with antipsychotics in rats as a model for antipsychotic-induced weight gain in human. *Pharmacology, Biochemistry and Behavior* 2003;75:133–40.
- [38] Ota M, Mori K, Nakashima A, Kaneko Y, Fujiwara K, Itoh M, et al. Peripheral injection of risperidone, an atypical antipsychotic, alters the body-weight gain of rats. *Clinical and Experimental Pharmacology and Physiology* 2002;29:980–9.
- [39] Thornton-Jones Z, Neill JC, Reynolds GP. The atypical antipsychotic olanzapine enhances ingestive behaviour in the rat: a preliminary study. *Journal of Psychopharmacology* 2002;16:35–7.
- [40] Hartfield A, Moore N, Clifton P. Effects of clozapine, olanzapine and haloperidol on the microstructure of ingestive behaviour in the rat. *Psychopharmacology* 2003;167:115.
- [41] Minet-Ringuet J, Even P, Lacroix M, Tomé D, de Beaupaire R. A model for antipsychotic-induced obesity in the male rat. *Psychopharmacology* 2006;187:447–54.
- [42] Shobo M, Yamada H, Mihara T, Kondo Y, Irie M, Harada K, et al. Two models for weight gain and hyperphagia as side effects of atypical antipsychotics in male rats: validation with olanzapine and ziprasidone. *Behavioural Brain Research* 2010;216:561–8.
- [43] Schleimer SB, Johnston GAR, Henderson JM. Novel oral drug administration in an animal model of neuroleptic therapy. *Journal of Neuroscience Methods* 2005;146:159–64.
- [44] Balcombe J, Barnard N, Sandusky C. Laboratory routines cause animal stress. *Contemporary Topics in Laboratory Animal Science* 2004;43:42–51.
- [45] Arjona AA, Zhang SX, Adamson B, Wurtman RJ. An animal model of antipsychotic-induced weight gain. *Behavioural Brain Research* 2004;152:121–7.
- [46] Fell M, Marshall K, Williams J, Neill J. Effects of the atypical antipsychotic olanzapine on reproductive function and weight gain in female rats. *Journal of Psychopharmacology* 2004;18:149–55.
- [47] Cooper G, Pickavance L, Wilding J, Halford J, Goudie A. A parametric analysis of olanzapine-induced weight gain in female rats. *Psychopharmacology* 2005;181:80–9.
- [48] Aravagiri M, Teper Y, Marder S. Pharmacokinetics and tissue distribution of olanzapine in rats. *Biopharmaceutics and Drug Disposition* 1999;20:369–77.
- [49] du Bois TM, Huang X-F, Deng C. Perinatal administration of PCP alters adult behaviour in female Sprague–Dawley rats. *Behavioural Brain Research* 2008;188:416–9.
- [50] Goudie A, Smith J, Halford J. Characterization of olanzapine-induced weight gain in rats. *Journal of Psychopharmacology* 2002;16:291–6.
- [51] Kalinichev M, Rourke C, Daniels A, Grizzle M, Britt C, Ignar D, et al. Characterisation of olanzapine-induced weight gain and effect of aripiprazole vs olanzapine on body weight and prolactin secretion in female rats. *Psychopharmacology* 2005;182:220–31.
- [52] Cope MB, Nagy TR, Fernandez JR, Geary N, Casey DE, Allison DB. Antipsychotic drug-induced weight gain: development of an animal model. *International Journal of Obesity and Related Metabolic Disorders* 2005;29:607–14.
- [53] Coccorello R, Caprioli A, Conti R, Ghirardi O, Borsini F, Carminati P, et al. Olanzapine (LY170053, 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5] benzodiazepine), but not the novel atypical antipsychotic ST2472 (9-piperazin-1-ylpyrrol[2,1-b][1,3]benzothiazepine), chronic administration induces weight gain, hyperphagia, and metabolic dysregulation in mice. *Journal of Pharmacology & Experimental Therapeutics* 2008;326:905–11.
- [54] Nasrallah H. A review of the effect of atypical antipsychotics on weight. *Psychoneuroendocrinology* 2003;28:83–96.
- [55] Nemeroff C. Dosing the antipsychotic medication olanzapine. *Journal of Clinical Psychiatry* 1997;58:45–9.
- [56] Gotthelf D, Falk B, Singer P, Kairi M, Phillip M, Zigel L, et al. Weight gain associated with increased food intake and low habitual activity levels in male adolescent schizophrenic inpatients treated with olanzapine. *American Journal of Psychiatry* 2002;159:1055–7.
- [57] Stefanidis A, Verty ANA, Allen AM, Owens NC, Cowley MA, Oldfield BJ. The role of thermogenesis in antipsychotic drug-induced weight gain. *Obesity* 2009;17:16–24.
- [58] Coccorello R, Brina D, Caprioli A, Conti R, Ghirardi O, Schepis F, et al. Days of continuous olanzapine infusion determines energy imbalance, glucose intolerance, insulin resistance, and dyslipidemia in mice. *Journal of Clinical Psychopharmacology* 2009;29:576–83. 510.1097/JCP.1090b1013e3181bf1013e.
- [59] Patil B, Kulkarni N, Unger B. Elevation of systolic blood pressure in an animal model of olanzapine induced weight gain. *European Journal of Pharmacology* 2006;551:112–5.
- [60] de Leon J, Verghese C, Josiassen R, Simpson G. Polydipsia and water intoxication in psychiatric patients: a review of the epidemiological literature. *Biological Psychiatry* 1994;35:519–30.
- [61] Lee H, Kwon K, Alphas L, Meltzer H. Effect of clozapine on psychogenic polydipsia in chronic schizophrenia. *Journal of Clinical Psychopharmacology* 1991;11:222.
- [62] Goldman MB, Hussain N. Absence of effect of olanzapine on primary polydipsia: results of a double-blind, randomized study. *Journal of Clinical Psychopharmacology* 2004;24:678–80.
- [63] Chintoh AF, Mann SW, Lam TKT, Giacca A, Remington G. Insulin resistance following continuous, chronic olanzapine treatment: an animal model. *Schizophrenia Research* 2008;104:23–30.
- [64] Schwartz MW, Woods SC, Porte D, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000;404:661–71.
- [65] Chiu C, Chen K, Liu H, Lu M. The early effect of olanzapine and risperidone on insulin secretion in atypical-naïve schizophrenic patients. *Journal of Clinical Psychopharmacology* 2006;26:504–7.
- [66] Chintoh AF, Mann SW, Lam L, Lam C, Cohn TA, Fletcher PJ, et al. Insulin resistance and decreased glucose-stimulated insulin secretion after acute olanzapine administration. *Journal of Clinical Psychopharmacology* 2008;28:494–9.
- [67] Wu R, Zhao J, Guo X, He Y, Fang M, Guo W, et al. Metformin addition attenuates olanzapine-induced weight gain in drug-naïve first-episode schizophrenia patients: a double-blind, placebo-controlled study. *American Journal of Psychiatry* 2008;165:352–8.
- [68] Chiu C-C, Chen C-H, Chen B-Y, Yu S-H, Lu M-L. The time-dependent change of insulin secretion in schizophrenic patients treated with olanzapine. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2010;34:866–70.
- [69] Bymaster FP, Nelson DL, Delapp NW, Falcone JF, Eckols K, Truex LL, et al. Antagonism by olanzapine of dopamine D1, serotonin2, muscarinic, histamine H1 and [alpha]1-adrenergic receptors in vitro. *Schizophrenia Research* 1999;37:107–22.
- [70] Johnson DE, Yamazaki H, Ward KM, Schmidt AW, Lebel WS, Treadway JL, et al. Inhibitory effects of antipsychotics on carbachol-enhanced insulin secretion from perfused rat islets: role of muscarinic antagonism in antipsychotic-induced diabetes and hyperglycemia. *Diabetes* 2005;54:1552–8.
- [71] Balakrishnan S, Mathew J, Antony S, Paulose CS. Muscarinic M1, M3 receptors function in the brainstem of streptozotocin induced diabetic rats: their role in insulin secretion from the pancreatic islets as a function of age. *European Journal of Pharmacology* 2009;608:14–22.
- [72] Renuka TR, Ani DV, Paulose CS. Alterations in the muscarinic M1 and M3 receptor gene expression in the brain stem during pancreatic regeneration and insulin secretion in weanling rats. *Life Sciences* 2004;75:2269–80.
- [73] Murashita M, Kusumi I, Hosoda H, Kangawa K, Koyama T. Acute administration of clozapine concurrently increases blood glucose and circulating plasma ghrelin levels in rats. *Psychoneuroendocrinology* 2007;32:777–84.
- [74] Murashita M, Kusumi I, Inoue T, Takahashi Y, Hosoda H, Kangawa K, et al. Olanzapine increases plasma ghrelin level in patients with schizophrenia. *Psychoneuroendocrinology* 2005;30:106–10.
- [75] Kim B-J, Sohn J-W, Park C-S, Hahn G-H, Koo J, Noh Y-D, et al. Body weight and plasma levels of ghrelin and leptin during treatment with olanzapine. *Journal of Korean Medical Science* 2008;23:685–90.
- [76] Hosojima H, Togo T, Odawara T, Hasegawa K, Miura S, Kato Y, et al. Early effects of olanzapine on serum levels of ghrelin, adiponectin and leptin in patients with schizophrenia. *Journal of Psychopharmacology* 2006;20:75–9.
- [77] Popovic V, Doknic M, Maric N, Pekic S, Damjanovic A, Miljic D, et al. Changes in neuroendocrine and metabolic hormones induced by atypical antipsychotics in normal-weight patients with schizophrenia. *Neuroendocrinology* 2007;85:249–56.
- [78] Berridge KC, Pecina S. Benzodiazepines, appetite, and taste palatability. *Neuroscience and Biobehavioral Reviews* 1995;19:121–31.
- [79] Hiejima H, Nishi Y, Hosoda H, Yoh J, Mifune H, Satou M, et al. Regional distribution and the dynamics of n-decanoyl ghrelin, another acyl-form of ghrelin, upon fasting in rodents. *Regulatory Peptides* 2009;156:47–56.
- [80] Orr J, Davy B. Dietary influences on peripheral hormones regulating energy intake: potential applications for weight management. *Journal of the American Dietetic Association* 2005;105:1115–24.

- [81] Shrestha YB, Wickwire K, Giraudo SQ. Direct effects of nutrients, acetylcholine, CCK, and insulin on ghrelin release from the isolated stomachs of rats. *Peptides* 2009;30:1187–91.
- [82] Burdyga G, Lal S, Varro A, Dimaline R, Thompson DG, Dockray GJ. Expression of Cannabinoid CB1 receptors by vagal afferent neurons is inhibited by cholecystokinin. *Journal of Neuroscience* 2004;24:2708–15.
- [83] Beinfeld MC, Garver DL. Concentration of cholecystokinin in cerebrospinal fluid is decreased in psychosis: relationship to symptoms and drug response. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 1991;15:601–9.
- [84] Bachus SE, Hyde TM, Herman MM, Egan MF, Kleinman JE. Abnormal cholecystokinin mRNA levels in entorhinal cortex of schizophrenics. *Journal of Psychiatric Research* 1997;31:233–56.
- [85] Virgo L, Humphries C, Mortimer A, Barnes T, Hirsch S, de Bellerocche J. Cholecystokinin messenger RNA deficit in frontal and temporal cerebral cortex in schizophrenia. *Biological Psychiatry* 1995;37:694–701.
- [86] Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, et al. Inhibition of food intake in obese subjects by Peptide YY(3–36). *New England Journal of Medicine* 2003;349:941–8.
- [87] Zwirski-Korczala K, Konturek S, Sadowski M, Wylezol M, Kuka D, Sowa P, et al. Basal and postprandial plasma levels of PYY, ghrelin, cholecystokinin, gastrin and insulin in women with moderate and morbid obesity and metabolic syndrome. *Journal of Physiology and Pharmacology* 2007;58:13–35.
- [88] Wang Q, Huang X-F. Effects of chronic treatment of olanzapine and haloperidol on peptide YY binding densities in the rat brain. *Experimental Neurology* 2008;209:261–7.
- [89] Beasley JCM, Tollefson G, Tran P, Satterlee W, Sanger T, Hamilton S. Olanzapine versus placebo and haloperidol: Acute phase results of the North American double-blind olanzapine trial. *Neuropsychopharmacology* 1996;14:111–23.
- [90] Sharpe J-K, Byrne NM, Stedman TJ, Hills AP. Resting energy expenditure is lower than predicted in people taking atypical antipsychotic medication. *Journal of the American Dietetic Association* 2005;105:612–5.
- [91] Virkkunen M, Wahlbeck K, Rissanen A, Naukkarinen H, Franssila-Kallunki A. Decrease of energy expenditure causes weight increase in olanzapine treatment—a case study. *Pharmacopsychiatry* 2002;35(124):126.
- [92] Procyshyn RM, Chau A, Tse G. Clozapine's effects on body weight and resting metabolic rate: a case series. *Schizophrenia Research* 2004;66:159–62.
- [93] Cope MB, Li X, Jumbo-Lucioni P, DiCostanzo CA, Jamison WG, Kesterson RA, et al. Risperidone alters food intake, core body temperature, and locomotor activity in mice. *Physiology and Behavior* 2009;96:457–63.
- [94] Karl T, Duffy L, O'Brien E, Matsumoto I, Dedova I. Behavioural effects of chronic haloperidol and risperidone treatment in rats. *Behavioural Brain Research* 2006;171:286–94.
- [95] Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *The FASEB Journal* 2007;22:659–61.
- [96] Cooper GD, Harrold JA, Halford JCG, Goudie AJ. Chronic clozapine treatment in female rats does not induce weight gain or metabolic abnormalities but enhances adiposity: implications for animal models of antipsychotic-induced weight gain. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2008;32:428–36.
- [97] Tulipano G, Rizzetti C, Bianchi I, Fanzani A, Spano P, Cocchi D. Clozapine-induced alteration of glucose homeostasis in the rat: the contribution of hypothalamic–pituitary–adrenal axis activation. *Neuroendocrinology* 2007;85:61.
- [98] Smith G, Chaussade C, Vickers M, Jensen J, Shepherd P. Atypical antipsychotic drugs induce derangements in glucose homeostasis by acutely increasing glucagon secretion and hepatic glucose output in the rat. *Diabetologia* 2008;51:2309–17.
- [99] Weston-Green K, Huang X-F, Deng C. Sensitivity of the female rat to olanzapine-induced weight gain—Far from the clinic? *Schizophrenia Research* 2010;116:299–300.
- [100] Ryan MCM, Flanagan S, Kinsella U, Keeling F, Thakore JH. The effects of atypical antipsychotics on visceral fat distribution in first episode, drug-naïve patients with schizophrenia. *Life Sciences* 2004;74:1999–2008.
- [101] van der Zwaal EM, Luijendijk MCM, Evers SS, la Fleur SE, Adan RAH. Olanzapine affects locomotor activity and meal size in male rats. *Pharmacology Biochemistry and Behavior* 2010;97(1):130–7.

CHAPTER THREE

EFFECTS OF OLANZAPINE ON MUSCARINIC M3 RECEPTOR BINDING DENSITY IN THE BRAIN RELATES TO WEIGHT GAIN, PLASMA INSULIN AND METABOLIC HORMONE LEVELS

Reprinted from European Neuropsychopharmacology, 22(5), Weston-Green K, Huang XF, Lian J, Deng C, Effects of olanzapine on muscarinic M3 receptor binding density in the brain related to weight gain, plasma insulin and metabolic hormone levels, 364-373, Copyright (2011), with permission from Elsevier.



ELSEVIER

www.elsevier.com/locate/euroneuro



Effects of olanzapine on muscarinic M3 receptor binding density in the brain relates to weight gain, plasma insulin and metabolic hormone levels

Katrina Weston-Green^{a, b}, Xu-Feng Huang^{a, b}, Jiamei Lian^a, Chao Deng^{a, b, *}

^a Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, Wollongong, 2522, NSW, Australia

^b Schizophrenia Research Institute, 384 Victoria Street, Darlinghurst, 2010, NSW, Australia

Received 30 April 2011; received in revised form 1 August 2011; accepted 1 September 2011

KEYWORDS

Antipsychotic agents;
Muscarinic M3 receptor;
Metabolism;
Diabetes mellitus;
Obesity

Abstract

The second generation antipsychotic drug (SGA) olanzapine has an efficacy to treat schizophrenia, but can cause obesity and type II diabetes mellitus. Cholinergic muscarinic M3 receptors (M3R) are expressed on pancreatic β -cells and in the brain where they influence insulin secretion and may regulate other metabolic hormones via vagal innervation of the gastrointestinal tract. Olanzapine's M3R antagonism is an important risk factor for its diabetogenic liability. However, the effects of olanzapine on central M3Rs are unknown. Rats were treated with 0.25, 0.5, 1.0 or 2.0 mg olanzapine/kg or vehicle (3 \times /day, 14-days). M3R binding densities in the hypothalamic arcuate (Arc) and ventromedial nuclei (VMH), and dorsal vagal complex (DVC) of the brainstem were investigated using [³H]4-DAMP plus pirenzepine and AF-DX116. M3R binding correlations to body weight, food intake, insulin, ghrelin and cholecystokinin (CCK) were analyzed. Olanzapine increased M3R binding density in the Arc, VMH and DVC, body weight, food intake, circulating plasma ghrelin and CCK levels, and decreased plasma insulin and glucose. M3R negatively correlated to insulin, and positively correlated to ghrelin, CCK, food intake and body weight. Increased M3R density is a compensatory up-regulation in response to olanzapine's M3R antagonism. Olanzapine acts on M3R in regions of the brain that control food intake and insulin secretion. Olanzapine's M3R blockade in the brain may inhibit the acetylcholine pathway for insulin secretion. These findings support a role for M3Rs in the modulation of insulin, ghrelin and CCK via the vagus nerve and provide a mechanism for olanzapine's diabetogenic and weight gain liability. © 2011 Elsevier B.V. and ECNP. All rights reserved.

* Corresponding author at: IHMRI, Building 32, University of Wollongong, Northfields Avenue, Wollongong, NSW 2522, Australia. Tel.: +61 2 4221 4934; fax: +61 2 4221 8130.

E-mail address: chao@uow.edu.au (C. Deng).

1. Introduction

The efficacy for second generation antipsychotic drugs (SGAs) to treat the positive, negative and cognitive symptoms of schizophrenia has led to their increased use in the clinic. However, some SGAs are associated with metabolic side-effects, including weight gain and glucoregulatory abnormalities such as insulin-resistance and type II diabetes mellitus (American Diabetes Association et al., 2004; Newcomer, 2005). Olanzapine has an efficacy to treat schizophrenia with enhanced tolerability compared to older first generation antipsychotics (Haro et al., 2009; Lieberman et al., 2005), but has a particularly high diabetogenic liability and risk for increasing body weight (Newcomer, 2005). Chronic olanzapine treatment is also associated with hyperinsulinemia, hepatic insulin resistance and type II diabetes mellitus in humans (Oriot et al., 2008; Perez-Iglesias et al., 2008), rats (Albaugh et al., 2006; Chintoh et al., 2008b) and mice (Coccurello et al., 2009), even independent of body weight gain (Chintoh et al., 2009; Ramankutty, 2002). On the other hand, decreased insulin levels have been reported following short-term olanzapine treatment i.e.: in humans following 14-day administration (Chiu et al., 2006), male rats after a single acute injection (Chintoh et al., 2008a), and from pancreatic β -cells in vitro (Johnson et al., 2005). Indeed, the incidence of obesity and type II diabetes mellitus (DM) in schizophrenia is 1.5–2.0 times higher than the general population (American Diabetes Association et al., 2004), highlighting the importance and urgency of understanding the mechanisms underlying SGA-induced metabolic side-effects.

The molecular pathways regulating glucose metabolism and body weight have become a target of interest in identifying the mechanisms of SGA-induced metabolic dysfunction (see reviews by Coccurello and Moles, 2010; Reynolds and Kirk, 2010). Central and peripheral cholinergic pathways play an important role in the normal maintenance of energy and glucose homeostasis, including regulation of gastroenteropancreatic functions via the vagus nerve (Berthoud, 2008; Dockray, 2009). The effects of acetylcholine (ACh) are mediated by the muscarinic receptor sub-types (M1–M5), with a recent interest generated in the muscarinic M3 receptor (M3R) due to its facilitation of the ACh pathway for insulin secretion and subsequent regulation of glucose homeostasis (Ruiz de Azua et al., 2011). The M3R is widely expressed in the brain, including the ventromedial hypothalamic (VMH) and arcuate (Arc) nuclei of the hypothalamus, and the dorsal vagal complex (DVC) of the brainstem (Levey et al., 1994; Zubieta and Frey, 1993), regions well-documented for their role in insulin and glucagon secretion, glucose homeostasis and body weight regulation (Buijs et al., 2001; Li et al., 2003; Renuka et al., 2004; Rohner-Jeanrenaud and Jeanrenaud, 1980). M3Rs are also abundantly expressed on pancreatic beta cells where they modulate the ACh pathway for insulin secretion (Gautam et al., 2006b). Antipsychotic affinity for the M3R is considered the best indicator for diabetogenic liability (Silvestre and Prous, 2005) and relates to weight gain risk (Matsui-Sakata et al., 2005). Interestingly, olanzapine and clozapine have a profile to potently block the M3R, while antipsychotics with a lower risk of metabolic dysfunction side-effect, such as risperidone and ziprasidone, have little effect on the M3R (Johnson et al.,

2005). Olanzapine and clozapine can decrease insulin secretion in vitro, while risperidone and ziprasidone lack effect on insulin (Johnson et al., 2005). Despite this significance, the effects of olanzapine on M3Rs in regions of the brain that influence insulin secretion are unknown. In addition, M3Rs may be important in the regulation of metabolic hormones such as ghrelin and CCK, via cholinergic vagal innervation to the stomach and intestine. However, studies on the role of the M3R in the regulation of olanzapine-induced metabolic side-effects are lacking. Therefore, in the present study we investigated the effects of olanzapine dosage on M3R density in the hypothalamus and brainstem, and examined the relationship between M3R binding data and insulin, ghrelin and cholecystokinin (CCK) levels, food intake and body weight gain. The present study provides the first evidence that olanzapine alters M3R signaling in regions of the brain that control insulin secretion and energy homeostasis.

2. Experimental procedures

2.1. Animals and diet

Female SD rats (7-weeks old, Animal Resources Centre, Perth, WA, Australia), were housed at 22 °C, on a 12-h light–dark cycle (lights on: 07:00 h), and allowed ad libitum access to water and standard laboratory chow diet (3.9 kcal/g; 10% fat, 74% carbohydrate, 16% protein) throughout the study. Following one week habituation, animals underwent a teaching period to self-administer a sweet cookie dough pellet three-times per day for one week. Animals were randomly assigned to one of the following treatments: 0.25, 0.5, 1.0 or 2.0 mg olanzapine/kg (Eli Lilly, Indianapolis, IN, USA), or vehicle ($n=12$), three times daily, self-administered orally in a sweet cookie-dough pellet for 14-days. Dosages were clinically relevant based on D2 receptor occupancy (Kapur et al., 2003), translated between species based on body surface area (Reagan-Shaw et al., 2007). Administration interval was 8-hourly to avoid fluctuations in drug levels in accordance with the half-life of olanzapine in the rat (Aravagiri et al., 1999). Cookie dough (62% carbohydrate, 22% protein, 6% fiber, 10% vitamins and minerals) administration methods were employed as previously reported (Weston-Green et al., 2011). Briefly, a mixture of cornstarch (30.9%), sucrose (30.9%), gelatine (6.3%), casein (15.5%), fiber (6.4%), minerals (8.4%) and vitamins (1.6%) was produced. The assigned dosage of powdered olanzapine was added to the measured dry ingredients. Water was added to achieve a dry-dough consistency immediately prior to administration and a 0.3 g cookie-dough pellet (containing the assigned olanzapine dosage) was offered by metal spoon. Daily body weight and food intake measurements were obtained (Weston-Green et al., 2011). All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

2.2. Hormonal measurements

As reported previously (Weston-Green et al., 2011), animals underwent a drug wash-out period of 10–12 h after 14-day olanzapine treatment, and were fasted for 4–6 h prior to euthanasia with a lethal intraperitoneal injection of sodium pentobarbitone. Commercially available ELISA kits were used to detect circulating insulin levels (Linco Research, Missouri, USA), and RIA kits were used to detect circulating levels of total ghrelin (Linco Research, MI, USA) and CCK (Phoenix Pharmaceuticals, CA, USA). Blood glucose was measured using a blood glucose monitor (Roche Diagnostics Group, Mannheim, Germany).

2.3. Histological procedures

Six brains were randomly selected from each treatment group for further analysis. Coronal sections were cut on a cryostat (14 μ m) at -18°C corresponding to the levels of Bregma -2.40 mm and -13.68 mm (Paxinos and Watson, 2007), which incorporated the Arc and VMH of the hypothalamus and the DVC of the brainstem. Sections were thaw-mounted onto PolysineTM Microscope Slides (Menzel GmbH & Co. KG, Braunschweig, Germany) and stored at -20°C .

2.4. M3R binding procedures

M3R binding methods were modified from Zubieta and Frey (1993). Briefly, slides were air-dried then pre-incubated for 5 min in PBS-EDTA buffer (137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4 , 1.5 mM KH_2PO_4 and 1 mM EDTA, pH 7.4) at room temperature. For total binding, sections were incubated for 60 min with the non-specific muscarinic receptor antagonist 10 nM [^3H]4-DAMP (65.1 Ci/mmol, PerkinElmer Life and Analytical Sciences, Boston MA), in the presence of 2 μM pirenzepine dihydrochloride and 2 μM AF-DX 116 dissolved in slightly warmed DMSO (Tocris Bioscience, Ellisville MO); the latter were used to minimize M1/M4 and M2/M4 receptor binding, respectively. Non-specific binding was determined by incubating

subsequent sections in 10 nM [^3H]4-DAMP, 2 μM pirenzepine dihydrochloride and 2 μM AF-DX 116, in the presence of 20 μM atropine sulfate. Sections were washed in PBS-EDTA (pH 7.4) four times for 30 s at 4°C , dipped in ice cold milliQ H_2O to remove buffer salts, then gently air dried in a stream of cool air.

2.5. Autoradiography and quantification of M3R binding

M3R autoradiographic images were captured using a high-resolution beta image camera (BioSpace, Paris, France), which determined the level of radioactivity bound to the brain tissue by directly counting the amount of β -particles emitted from the brain sections, as we have previously described (Deng et al., 2007). After 3.5 hour exposure, radioactive levels were provided in counts per minute per square millimeter of tissue (cpm/ mm^2) and were converted to nCi/mg tissue equivalent using standard tissue sections calibrated with commercial standards (Amersham, Buckinghamshire, United Kingdom) (1.4–33.8 nCi/mg tissue equivalent). Taking into account the specific activity of the ligand [^3H]4-DAMP (65.1 Ci/mmol), final radioligand binding measurements were transformed into fmol/mg tissue equivalent. Quantification was performed using β -Image Plus software (version 4, BioSpace) at the Bregma levels -2.40 mm and -13.68 mm, incorporating the Arc, VMH and DVC. Nuclei of interest were confirmed using a corresponding set of cresyl violet-stained slides and a standard rat brain atlas (Paxinos and Watson, 2007).

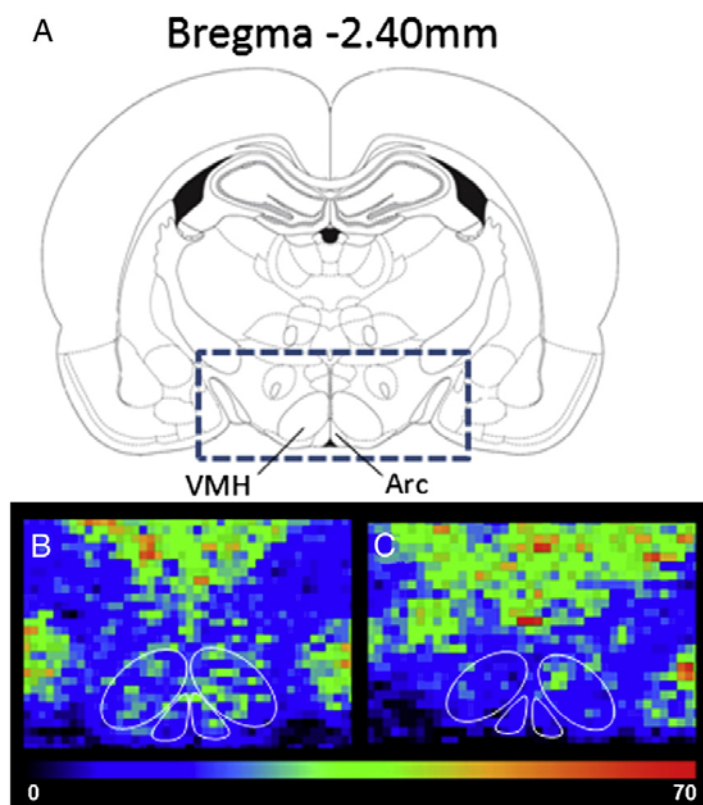


Figure 1 Example of digital autoradiogram obtained with a Beta Imager to show muscarinic M3 receptor binding. (A) The schematic diagram modified from a rat brain atlas (Paxinos and Watson, 2007) showing the level of Bregma -2.40 mm. M3 receptor binding in the ventromedial hypothalamic (VMH) and arcuate (Arc) nuclei of the hypothalamus following (B): 2.0 mg/kg olanzapine or (C): vehicle (control) for 14-days t.i.d. Scale bar: 0–70 fmol/mg tissue equivalent.

2.6. Statistical analysis

Data were statistically analyzed using SPSS (version 17, SPSS, Chicago, IL, USA). One-way ANOVAs were employed to determine the dosage response of olanzapine on M3R binding density, blood glucose levels, plasma insulin and CCK levels, total body weight and food intake, while total ghrelin levels were analyzed using Kruskal–Wallis tests followed by Mann–Whitney U post-hoc analysis. All other post-hoc comparisons were performed using Dunnett–T tests. Correlations were examined using Pearson's correlation tests or Spearman's correlation tests for non-parametric data. Data was considered significant when $p < 0.05$.

3. Results

3.1. M3R binding

An example of M3R binding is presented in Fig. 1. An ANOVA revealed a significant effect of olanzapine dosage on M3R binding density in the Arc ($F_{4,25} = 9.94$, $p < 0.01$), DVC ($F_{4,25} = 9.31$, $p < 0.01$) and VMH ($F_{4,25} = 13.46$, $p < 0.01$). Post-hoc analysis identified a significant increase in M3R binding in the Arc following 0.5, 1.0 and 2.0 mg/kg ($p < 0.01$) olanzapine treatment, while the low dosage of 0.25 mg/kg olanzapine showed a trend toward significance ($p = 0.053$) compared to controls (Fig. 2A). M3R binding density increased in the VMH in all treatment groups (0.25 mg/kg, $p < 0.05$; 0.5–2.0 mg/kg, $p < 0.01$) compared to controls (Fig. 2B). Olanzapine increased M3R binding density in the DVC of animals treated with 0.5 mg/kg ($p < 0.05$), 1.0 mg/kg and 2.0 mg/kg ($p < 0.01$) olanzapine, but not after 0.25 mg/kg olanzapine treatment ($p > 0.05$) (Fig. 2C).

3.2. Insulin, glucose and appetite hormonal levels, weight gain and food intake

Olanzapine treatment significantly increased total body weight gain in animals treated with 0.5 mg/kg, 1.0 mg/kg ($p < 0.05$) and 2.0 mg/kg olanzapine ($p < 0.01$), but not 0.25 mg/kg ($p > 0.05$), compared to controls (Fig. 3A). Total cumulative food intake increased after 2.0 mg/kg olanzapine only ($p < 0.01$) (Fig. 3B). Plasma insulin significantly decreased following 0.25, 0.5, 1.0 (all $p < 0.05$) and 2.0 mg/kg ($p < 0.01$) olanzapine treatment and total ghrelin significantly increased in all dosage groups ($p < 0.05$ for 0.25, 1.0 and 2.0 mg/kg olanzapine, $p < 0.01$ for 0.5 mg/kg olanzapine treatment groups) compared to controls (Fig. 3C and D, respectively). CCK increased following 0.5 mg/kg and 2.0 mg/kg olanzapine ($p < 0.01$), but unexpectedly not in animals treated with 1.0 mg/kg olanzapine compared to controls ($p > 0.05$) (Fig. 3E). Additionally, there was a significant decrease in blood glucose levels following 0.5 mg/kg (–24%, $p < 0.05$), 1.0 mg/kg (–25%, $p < 0.05$) and 2.0 mg/kg (–28%, $p < 0.01$) olanzapine treatment for 14-days, but not in the low dosage 0.25 mg/kg treatment group (–10%, $p > 0.05$) (Fig. 3F).

3.3. Correlations

Insulin negatively correlated to M3R binding density in the Arc ($r = -0.46$, $p < 0.05$) (Fig. 4A), with a trend to correlate with M3R binding density in the VMH and DVC ($r = -0.34$, $p = 0.08$ and $r = -0.33$, $p = 0.09$, respectively). Ghrelin positively correlated to M3R binding density in the Arc ($r = 0.48$, $p < 0.05$), VMH ($r = 0.54$, $p < 0.05$) and DVC ($r = 0.61$, $p < 0.01$). CCK also correlated to M3R binding

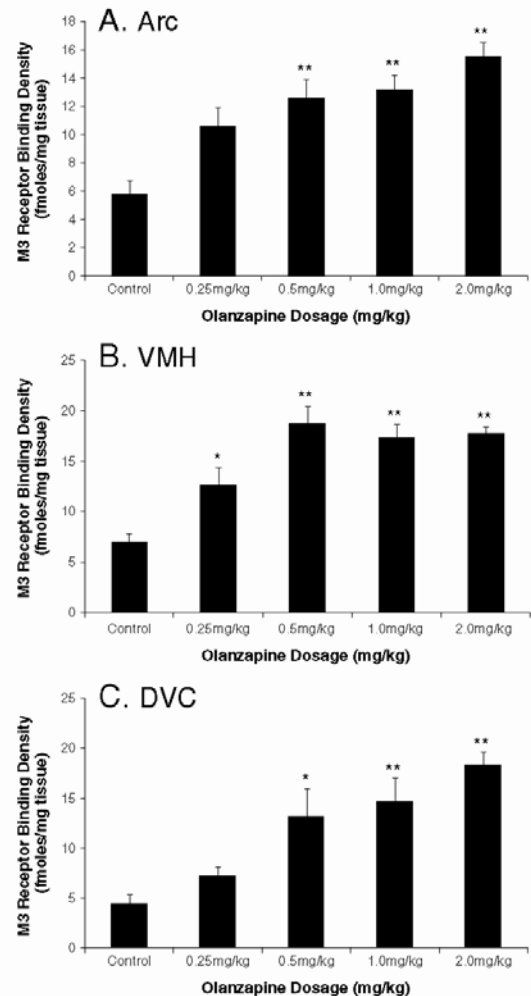


Figure 2 Muscarinic M3 receptor binding density in the A. arcuate nucleus, B: ventromedial hypothalamic nucleus, C: dorsal vagal complex (fmol/mg tissue equivalent mean \pm SEM) in female Sprague Dawley rats treated orally with 0.25, 0.5, 1.0 and 2.0 mg/kg olanzapine or control (vehicle) for 14-days ($n = 6$ /group). * $p < 0.05$, ** $p < 0.01$ vs. control.

density in the Arc ($r = 0.42$, $p < 0.05$), VMH ($r = 0.45$, $p < 0.05$) and DVC ($r = 0.53$, $p < 0.01$). A significant positive correlation was observed between total cumulative weight gain and M3R binding density in the Arc ($r = 0.40$, $p < 0.05$, Fig. 4B) and DVC ($r = 0.51$, $p < 0.01$), but not the VMH ($p = 0.10$). Similar correlations between total cumulative food intake and M3R binding density in the Arc ($r = 0.36$, $p < 0.05$) and DVC ($r = 0.46$, $p < 0.05$) were identified (Fig. 4C and D, respectively), but not in the VMH ($r = 0.26$, $p = 0.16$).

4. Discussion

The present paper provides the first evidence that olanzapine, an antipsychotic drug with a high diabetogenic and weight

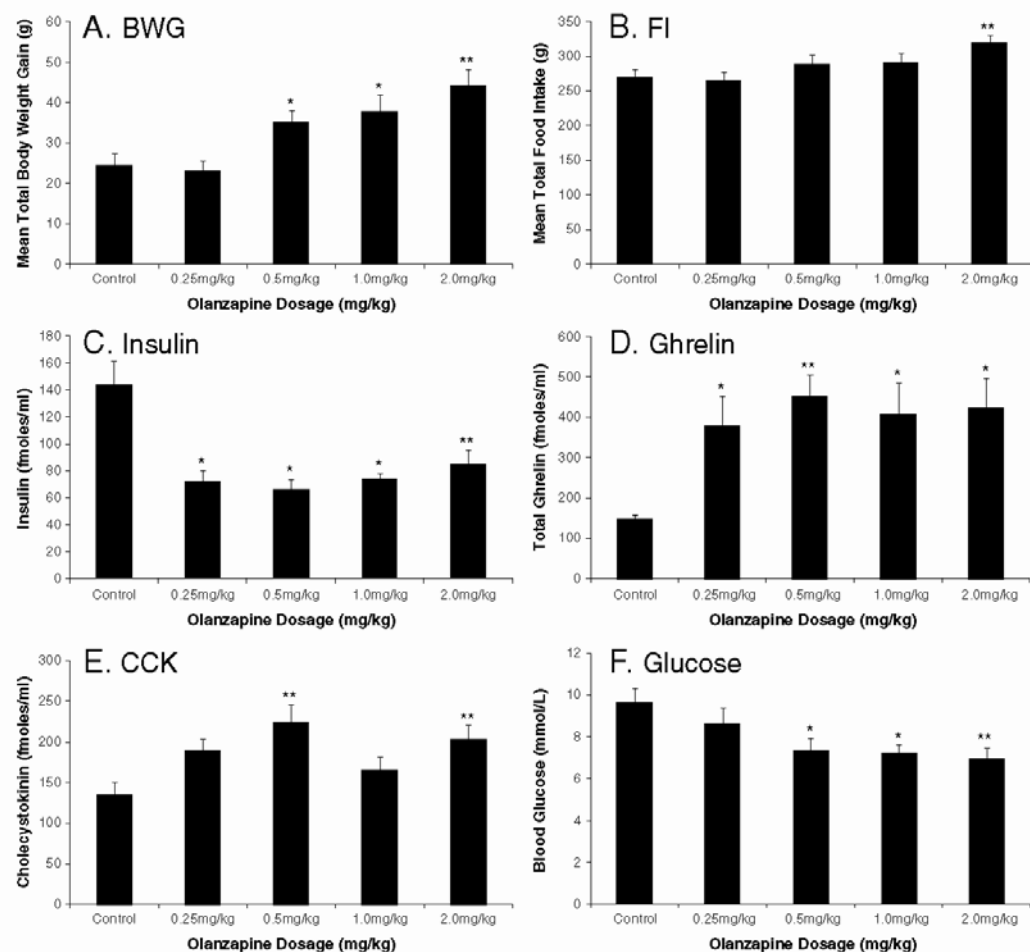


Figure 3 A: Mean total body weight gain (BWG) (g), B: mean total food intake (FI) (g), C: insulin (fmol/ml), D: total ghrelin (fmol/ml), E: cholecystokinin (CCK) (fmol/ml), F: blood glucose (mmol/L) in female Sprague Dawley rats treated with 0.25, 0.5, 1.0, 2.0 mg/kg olanzapine (self-administered orally, 3×/day for 14-days, n=12/group). Data is expressed as mean±SEM. * $p < 0.05$, ** $p < 0.01$ vs. control.

gain liability, alters M3R binding density in regions of the brain that regulate insulin and respond to metabolic hormonal signals to control energy homeostasis. Olanzapine significantly increased M3R binding density in the hypothalamic Arc and VMH, and the DVC of the brainstem. Coinciding with our weight gain data, a dosage of 0.5 mg/kg olanzapine was sufficient to induce significant changes in M3R binding density in the Arc, VMH and DVC, while the low dosage of 0.25 mg/kg olanzapine significantly increased M3R binding density in the VMH only, with a trend toward an increase in the Arc. Interestingly, M3R binding density was at a similar level in the 0.5, 1 and 2 mg/kg olanzapine-treated groups in the VMH. In the Arc and DVC, although the data displayed a general trend toward an increase in M3R binding density with increased olanzapine dosage from 0.5 to 2 mg/kg, the differences between these dosage groups were not significant. Therefore,

M3R binding density appeared to reach a maximal response threshold following 0.5 mg/kg olanzapine. Olanzapine induced hypoinsulinemia in all dosage groups following 14-days treatment, and M3R binding density in the brain was negatively associated with plasma insulin levels. This result was not surprising as activation of the M3R in the brain and pancreas stimulates ACh-mediated insulin secretion (Balakrishnan et al., 2009; Gautam et al., 2006b; Ruiz de Azua et al., 2011), however olanzapine potently blocks the M3R (Johnson et al., 2005). Therefore, olanzapine may cause insulin dysregulation by selectively impairing M3R signaling pathways in the brain and impacting the cholinergic pathway of insulin production. In addition, we found that M3R binding density correlated to increased ghrelin, CCK, food intake and body weight, following olanzapine treatment. Taken together, the present study suggests a potential role for central M3Rs in the regulation

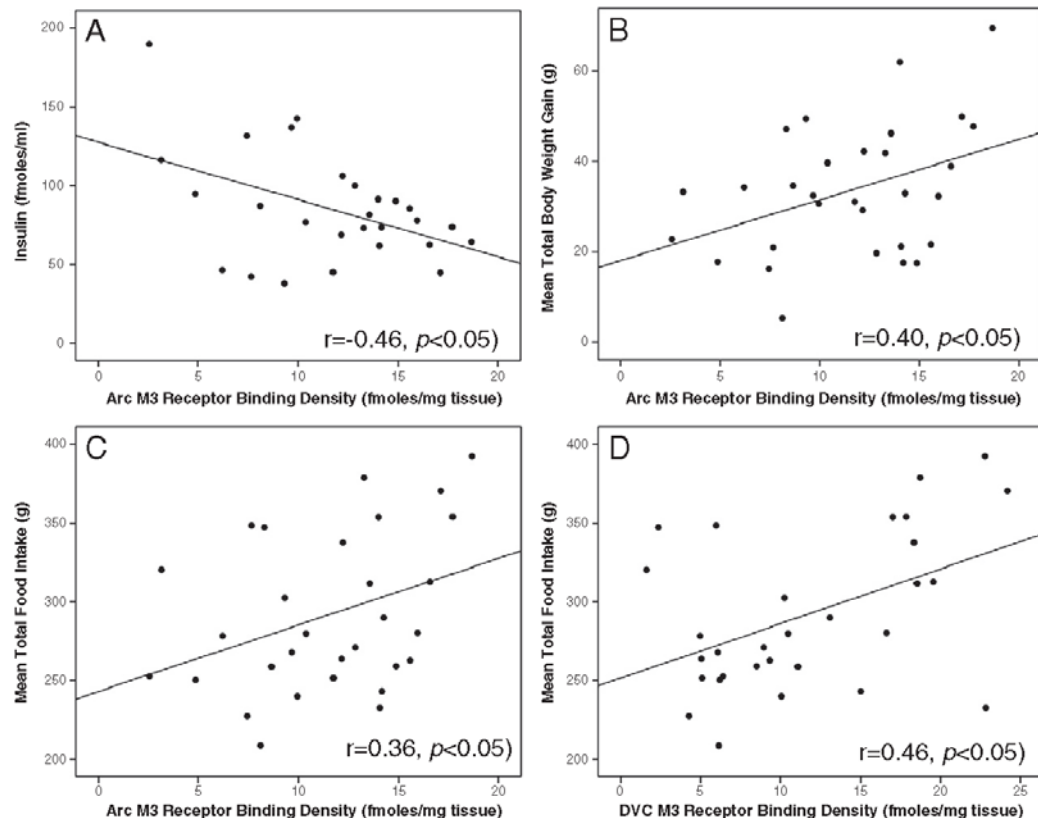


Figure 4 Correlations between M3 receptor binding density in the arcuate nucleus (Arc) and (A) insulin, (B) total body weight gain (g), (C) total food intake (g), and correlations between M3 receptor binding density in the dorsal vagal complex of the brainstem (DVC) and (D) total food intake (g), in female Sprague-Dawley rats orally self-administered olanzapine (14-days, 3×/day, n=6/group).

of metabolic hormones. Olanzapine's antagonism of the M3R may be a mechanism for its glucose dysfunction and, in-part, its weight gain side-effects.

The increased central M3R binding density observed in the present study may be a compensatory up-regulation in response to olanzapine's M3R blockade. Olanzapine's interference in M3R signaling in regions of the brain that influence pancreatic insulin secretion fits well with the high risk of olanzapine to induce insulin-resistance and diabetes. The drug's antagonistic effects on the M3R are likely to contribute to the hypoinsulinemia observed in the olanzapine-treated animals in the present study. Interestingly, olanzapine also decreased fasting blood glucose levels. This finding may be a result of insulin hypersensitivity due to low insulin levels. Hypoinsulinemia following 14-day olanzapine treatment may result in a compensatory up-regulation of insulin receptors in the body in order to maximize insulin response and attempt to maintain homeostasis, therefore glucose uptake/-metabolism would become more effective, causing low circulating glucose levels. Our results coincide with M3R knock-out studies where mutant mice with a global M3R-deficiency (M3R-KO) exhibit marked decreases in plasma insulin, glucose and glucagon levels (Duttaroy et al., 2004; Yamada et al., 2001). Mice lacking functional M3Rs specifically on

pancreatic beta cells (β -M3-KO) also manifest lower fasting insulin and glucose levels, and reduced glucose tolerance, whereas mice over-expressing M3Rs on pancreatic beta cells exhibit enhanced glucose tolerance and insulin release (Gautam et al., 2006b). Transgenic mice with chronically activated pancreatic β -cell M3R have enhanced insulin secretion (Gautam et al., 2010). Indeed, chronic olanzapine treatment increases plasma insulin levels in humans (Oriot et al., 2008; Perez-Iglesias et al., 2008), rats (Albaugh et al., 2006; Chintoh et al., 2008b) and mice (Coccurello et al., 2009). However, short-term olanzapine treatment decreases glucose-stimulated insulin secretion in individuals with schizophrenia after 14-day treatment (Chiu et al., 2006), and a single olanzapine dose decreased insulin secretory response following a glucose challenge in male rats (Chintoh et al., 2008a). In addition, olanzapine and clozapine attenuated glucose and cholinergic-stimulated insulin secretion from pancreatic islet cells in vitro, but not risperidone and ziprasidone, SGAs with a lower metabolic impact and low affinity for M3Rs (Johnson et al., 2005). These findings suggest that insulin is influenced by drug treatment period, i.e.: a time-dependent response of insulin secretion to olanzapine treatment. A time-related insulin hypothesis was recently evidenced in the clinic by Chiu et al. (2010), who reported hypoinsulinemia following a glucose-

challenge in people with schizophrenia following 14-day olanzapine treatment that returned to basal levels by week 4 of treatment, then increased by week 8 of treatment. Under normal conditions post-prandial hyperglycemia stimulates parasympathetic secretion of ACh from the vagus nerve, which activates M3Rs and subsequent insulin secretion (Gilon and Henquin, 2001). Preganglionic parasympathetic fibers arise from the DVC (Gilon and Henquin, 2001), which is influenced by the hypothalamus (Cone et al., 2001). We propose that olanzapine's blockade of M3Rs in specific regions of the brain, and on pancreatic beta cells (Johnson et al., 2005), decreases insulin secretion, which may induce insulin-hypersensitivity and increase glucose clearance in the short-term. Hypoglycemia stimulates food intake and excessive feeding may exaggerate insulin secretion during chronic olanzapine treatment as the body tries to establish glucose homeostasis, leading to hyperinsulinemia, insulin receptor desensitization and insulin-resistance. Impaired insulin secretion and glucose clearance are key pathogenic characteristics of type II diabetes mellitus (Bouche et al., 2004). This theory coincides with aspects of previous reviews (Silvestre and Prous, 2005; Starrenburg and Bogers, 2009) that have hypothesized a link between antipsychotic-induced M3R blockade and dysregulation of insulin metabolism as an important contributing factor to type II diabetes mellitus side-effects. Importantly, in the present study hypoinsulinemia was observed in animals treated with 0.25 mg/kg olanzapine that did not attain significant body weight gain, suggesting a direct drug effect on insulin levels independent of weight gain/obesity in this treatment group.

To our knowledge, the effects of M3R signaling pathways on ghrelin and CCK have not been investigated, however cholinergic vagal innervation from the brain to the stomach and intestine suggests a potential M3R role in the regulation of gastrointestinal hormones. In the present study, M3R binding density in the brain correlated to alterations in plasma ghrelin and CCK levels, suggesting that olanzapine's blockade of M3R signaling pathways induced metabolic hormonal changes that promote hyperphagia and weight gain. Coinciding with our theory, truncal vagotomy increases plasma ghrelin in humans and rats (Lee et al., 2002), and ghrelin secretion is increased during fasting while vagal parasympathetic activity is low, suggesting that the vagus nerve exerts an inhibitory tone over ghrelin (Lee et al., 2002; Toshinai et al., 2001). Vagal stimulation may even benefit metabolic syndrome management (Das, 2010). Olanzapine's blockade of the M3R may remove vagal inhibitory tone over ghrelin secretion leading to hyperphagia and body weight gain. Olanzapine-induced alterations in M3R binding density in the DVC and Arc (but not the VMH) were associated with food intake and weight gain. Vagal fibers innervating the gastrointestinal tract arise in the DVC (Berthoud, 2008). The DVC projects to the Arc, however the Arc can exert its own influence over gastrointestinal function directly via vagal efferent pathways (Berthoud, 2008; Tebbe et al., 2001). Olanzapine treatment resulted in increased circulating anorexigenic CCK concentrations, which may be a compensatory response elicited by the body to correct olanzapine-induced body weight gain by inhibiting food intake. Although vagotomy increases CCK levels (Liddle, 2000), vagal-cholinergic inhibition decreases the satiating effects of CCK (Smith et al., 1981), illustrating that CCK secretion and anorexigenic action are influenced by the

vagus nerve. Therefore, olanzapine's blockade of cholinergic M3R signaling pathways may increase CCK secretion, but interferes with the pathways for CCK satiation.

Seemingly contradictory to our findings, M3R-KO mice are hypophagic and lean (Duttaroy et al., 2004; Yamada et al., 2001), however M3Rs are widely distributed throughout the brain and body, and are involved in numerous functions (Ochi et al., 2005; Yang et al., 2009). Therefore, proper interpretation of metabolic differences between M3R-KO mice and olanzapine-induced M3R antagonism in the rat may be complicated by potential confounds attributed to whole-body M3R deficiency. It is interesting that both M3R-KO mice and β -M3-KO mice are lean and hypoinsulinemic (Duttaroy et al., 2004; Gautam et al., 2006a; Yamada et al., 2001), as insulin is anorexigenic, acting on the brain to suppress feeding by inhibiting the potent orexigen neuropeptide Y (NPY) in the Arc. Insulin-deficient rodents exhibit hyperphagia and weight gain (White et al., 1990), and hypoglycemia stimulates ghrelin mRNA expression in the stomach (Toshinai et al., 2001). Therefore, the hypo-insulinemic and -glycemic state of olanzapine-treated animals in the present study may also encourage hyperphagia and a positive weight balance.

Due to the current lack of a highly selective ligand, M3R binding density was examined in the present study using 4-DAMP, a muscarinic receptor antagonist with a high affinity for the M3R (Michel et al., 1989), as previously reported (Balakrishnan et al., 2009; Gibbons et al., 2009; Shen and Johnson, 2000). Based on the findings of Zubieta and Frey (1993), binding of 4-DAMP to other muscarinic receptor sub-types was blocked by concurrent use of pirenzepine dihydrochloride and AF-DX 116, which allowed competitive blockade of M1 and M2 receptors, respectively, with an approximate selectivity of 9:1 for M3 to M1 receptors, and 12:1 for M3 to M2 receptors. Olanzapine treatment may have affected other muscarinic receptor sub-types in the brain, however, to our knowledge effects in the periphery are yet to be elucidated. For example, olanzapine increased M1 mRNA expression in the hippocampus and substantia nigra (Han et al., 2008). Haloperidol and clozapine treatments increased [³H]pirenzepine binding in the frontal cortex (Crook et al., 2001). Olanzapine increased M1 and M2 receptor binding densities in a number of brain regions (although the hypothalamus and brainstem were not examined) (Terry et al., 2006). Clozapine and haloperidol increased M1 receptor binding in the hippocampus, while haloperidol decreased M2 receptor binding density in the amygdala (Zavitsanou et al., 2007). These effects on M1 and M2 may be related to the therapeutic effects of antipsychotics (Han et al., 2008). Only one study found that olanzapine decreased muscarinic M2 receptor binding density in the dorsal vagal complex that related to weight gain (Deng et al., 2007). Current literature suggests that the M3R is the sole receptor sub-type implicated in insulin production and secretion (Gilon and Henquin, 2001; Ruiz de Azua et al., 2011). In addition, the M3R has been identified as a predictor of antipsychotic diabetogenic liability (Silvestre and Prous, 2005) and may influence the level of other metabolic hormones.

In conclusion, we provide the first evidence that olanzapine, a potent antagonist of M3Rs, increases the binding density of M3Rs in key regions of the brain associated with body weight and insulin regulation. Our results suggest

that a possible mechanism for olanzapine-induced insulin dysfunction is through olanzapine's direct blockade of the M3R (i.e.: can be independent of weight gain) in the hypothalamus and the DVC of the brainstem, decreasing the cholinergic pathway for insulin secretion leading to later development of type II diabetes mellitus. Olanzapine's profile to potentially block the cholinergic M3R may remove tonic inhibition of ghrelin secretion and obstruct CCK satiety pathways via the vagus nerve, contributing to hyperphagia and body weight gain. Therefore, M3Rs may play an important role in the olanzapine-induced modulation of metabolic hormones. However, olanzapine treatment was associated with hypoinsulinemia and hyperghrelinemia, independent of dosage, whereas M3R binding density in the hypothalamus and DVC showed some dosage sensitivity. Investigation into the dosage effects of olanzapine on peripheral M3Rs would also be important as they may contribute to the lack of dosage sensitivity observed in some parameters measured in this study. For example, M3Rs expressed in the pancreas play a role in modulating insulin levels (Ruiz de Azua et al., 2011) and those expressed in white adipose tissue influence insulin-stimulated glucose uptake and glycerol release from adipocytes (Yang et al., 2009), whereas hepatic M3Rs do not appear to play a major role in glucose homeostasis (Li et al., 2009). Further studies into glucose tolerance and insulin sensitivity following an acute to chronic olanzapine treatment time-course and correlating to M3R effects will also be useful. Our data suggest an M3R contribution to olanzapine-induced body weight gain, however other receptors may play an important role, e.g.: the histaminergic H1 receptor has been identified as the main predictor for antipsychotic-induced body weight gain (Kroeze et al., 2003). The M3R may be more important for olanzapine-induced glucometabolic dysfunction and the development of type II diabetes mellitus. Our data shows that olanzapine acts on M3Rs in regions of the brain that control food intake and insulin secretion, supporting a role for the M3R in the modulation of metabolic hormones possibly via the vagus nerve. The M3R is of considerable therapeutic interest in terms of metabolic side-effects of olanzapine and other SGAs, and potential pharmacological treatments for diabetes and obesity.

Role of the funding source

Funding for this study was provided by a University of Wollongong grant to C Deng, an Australian National Health and Medical Research Council (NHMRC) grant to XF Huang and C Deng, and by the Schizophrenia Research Institute, Australia, utilizing infrastructure funding from NSW Health. The funding bodies had no further role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

Authors KWG, CD and XFH designed the study, authors KWG and JL carried out experimental procedures, author KWG performed statistical analysis, authors KWG, CD and XFH interpreted the results, author KWG wrote the first draft of manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

C. Deng has received honorarium from Eli Lilly Australia for presenting at the Cutting Edge Debate Melbourne (2010). The authors declare that there are no conflicts of interest.

Acknowledgments

We sincerely thank Dr Kai Kang for providing technical assistance.

References

- Albaugh, V., Henry, C., Bello, N., Hajnal, A., Lynch, S., Halle, B., Lynch, C., 2006. Hormonal and metabolic effects of olanzapine and clozapine related to body weight in rodents. *Obesity* 14, 36–50.
- American Diabetes Association, American Psychiatric Association, American Association of Clinical Endocrinologists, Obesity, N.A.A.f.t.S.o., 2004. Consensus development conference on antipsychotic drugs and obesity and diabetes. *Diab. Care* 27, 596–601.
- Australian Government National Health and Medical Research Council, 2004. Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, seventh ed. Australian Government, Canberra.
- Aravagiri, M., Teper, Y., Marder, S.R., 1999. Pharmacokinetics and tissue distribution of olanzapine in rats. *Biopharm. Drug Dispos.* 20, 369–377.
- Balakrishnan, S., Mathew, J., Antony, S., Paulose, C.S., 2009. Muscarinic M1, M3 receptors function in the brainstem of streptozotocin induced diabetic rats: their role in insulin secretion from the pancreatic islets as a function of age. *Eur. J. Pharmacol.* 608, 14–22.
- Berthoud, H.-R., 2008. The vagus nerve, food intake and obesity. *Regul. Pept.* 149, 15–25.
- Bouche, C., Serdy, S., Kahn, C.R., Goldfine, A.B., 2004. The cellular fate of glucose and its relevance in Type 2 diabetes. *Endocr. Rev.* 25, 807–830.
- Buijs, R., Chun, S., Nijima, A., Romijn, H., Nagai, K., 2001. Parasympathetic and sympathetic control of the pancreas: a role for the suprachiasmatic nucleus and other hypothalamic centers that are involved in the regulation of food intake. *J. Comp. Neurol.* 431, 405–423.
- Chintoh, A.F., Mann, S.W., Lam, L., Giacca, A., Fletcher, P., Nobrega, J., Remington, G., 2009. Insulin resistance and secretion in vivo: effects of different antipsychotics in an animal model. *Schizophr. Res.* 108, 127–133.
- Chintoh, A.F., Mann, S.W., Lam, L., Lam, C., Cohn, T.A., Fletcher, P.J., Nobrega, J.N., Giacca, A., Remington, G., 2008a. Insulin resistance and decreased glucose-stimulated insulin secretion after acute olanzapine administration. *J. Clin. Psychopharmacol.* 28, 494–499.
- Chintoh, A.F., Mann, S.W., Lam, T.K.T., Giacca, A., Remington, G., 2008b. Insulin resistance following continuous, chronic olanzapine treatment: an animal model. *Schizophr. Res.* 104, 23–30.
- Chiu, C.-C., Chen, C.-H., Chen, B.-Y., Yu, S.-H., Lu, M.-L., 2010. The time-dependent change of insulin secretion in schizophrenic patients treated with olanzapine. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 34, 866–870.
- Chiu, C., Chen, K., Liu, H., Lu, M., 2006. The early effect of olanzapine and risperidone on insulin secretion in atypical-naïve schizophrenic patients. *J. Clin. Psychopharmacol.* 26, 504–507.
- Coccarello, R., Brina, D., Caprioli, A., Conti, R., Ghirardi, O., Schepis, F., Moles, A., 2009. 30 days of continuous olanzapine infusion determines energy imbalance, glucose intolerance,

- insulin resistance, and dyslipidemia in mice. *J. Clin. Psychopharmacol.* 29, 576–583.
- Coccurello, R., Moles, A., 2010. Potential mechanisms of atypical antipsychotic-induced metabolic derangement: clues for understanding obesity and novel drug design. *Pharmacol. Ther.* 127, 210–251.
- Cone, R.D., Cowley, M.A., Butler, A.A., Fan, W., Marks, D.L., Low, M.J., 2001. The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. *Int. J. Obes. Relat. Disord.* 25, S63.
- Crook, J.M., Tomaskovic-Crook, E., Copolov, D.L., Dean, B., 2001. Low muscarinic receptor binding in prefrontal cortex from subjects with schizophrenia: a study of Brodmann's areas 8, 9, 10, and 46 and the effects of neuroleptic drug treatment. *Am. J. Psychiatry* 158, 918–925.
- Das, U.N., 2010. Vagus nerve stimulation as a strategy to prevent and manage metabolic syndrome. *Med. Hypotheses* 76, 429–433.
- Deng, C., Weston-Green, K.L., Han, M., Huang, X.-F., 2007. Olanzapine treatment decreases the density of muscarinic M2 receptors in the dorsal vagal complex of rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 31, 915–920.
- Dockray, G.J., 2009. The versatility of the vagus. *Physiol. Behav.* 97, 531–536.
- Duttaroy, A., Zimlik, C.L., Gautam, D., Cui, Y., Mears, D., Wess, J., 2004. Muscarinic stimulation of pancreatic insulin and glucagon release is abolished in [M.sub.3] muscarinic acetylcholine receptor-deficient mice. (Islet studies). *Diabetes* 53, 1714–1717.
- Gautam, D., de Azua, I.R., Li, J.H., Guettier, J.-M., Heard, T., Cui, Y., Lu, H., Jou, W., Gavrilova, O., Zawulich, W.S., Wess, J., 2010. Beneficial metabolic effects caused by persistent activation of [beta]-cell M3 muscarinic acetylcholine receptors in transgenic mice. *Endocrinology* 151, 5185–5194.
- Gautam, D., Gavrilova, O., Jeon, J., Pack, S., Jou, W., Cui, Y., Li, J.H., Wess, J., 2006a. Beneficial metabolic effects of M3 muscarinic acetylcholine receptor deficiency. *Cell Metab.* 4, 363–375.
- Gautam, D., Han, S.-J., Hamdan, F.F., Jeon, J., Li, B., Li, J.H., Cui, Y., Mears, D., Lu, H., Deng, C., Heard, T., Wess, J., 2006b. A critical role for [beta] cell M3 muscarinic acetylcholine receptors in regulating insulin release and blood glucose homeostasis in vivo. *Cell Metab.* 3, 449–461.
- Gibbons, A.S., Scarr, E., McLean, C., Sundram, S., Dean, B., 2009. Decreased muscarinic receptor binding in the frontal cortex of bipolar disorder and major depressive disorder subjects. *J. Affect. Disord.* 116, 184–191.
- Gilon, P., Henquin, J.-C., 2001. Mechanisms and physiological significance of the cholinergic control of pancreatic [beta]-cell function. *Endocr. Rev.* 22, 565–604.
- Han, M., Newell, K., Zavitsanos, K., Deng, C., Huang, X.-F., 2008. Effects of antipsychotic medication on muscarinic M1 receptor mRNA expression in the rat brain. *J. Neurosci. Res.* 86, 457–464.
- Haro, J.M., Novick, D., Suarez, D., Roca, M., 2009. Antipsychotic treatment discontinuation in previously untreated patients with schizophrenia: 36-month results from the SOHO study. *J. Psychiatr. Res.* 43, 265–273.
- Johnson, D.E., Yamazaki, H., Ward, K.M., Schmidt, A.W., Lebel, W.S., Treadway, J.L., Gibbs, E.M., Zawulich, W.S., Rollema, H., 2005. Inhibitory effects of antipsychotics on carbachol-enhanced insulin secretion from perfused rat islets: role of muscarinic antagonism in antipsychotic-induced diabetes and hyperglycemia. *Diabetes* 54, 1552–1558.
- Kapur, S., Vanderspek, S.C., Brownlee, B.A., Nobrega, J., 2003. Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: a suggested solution based on in vivo occupancy. *J. Pharmacol. Exp. Ther.* 305, 625–631.
- Kroeze, W.K., Hufeisen, S.J., Popadak, B.A., Renock, S.M., Steinberg, S., Emsberger, P., Jayathilake, K., Meltzer, H.Y., Roth, B.L., 2003. H1-histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. *Neuropsychopharmacology* 28, 519–526.
- Lee, H.-M., Wang, G., Englander, E.W., Kojima, M., Greeley Jr., G.H., 2002. Ghrelin, a new gastrointestinal endocrine peptide that stimulates insulin secretion: enteric distribution, ontogeny, influence of endocrine, and dietary manipulations. *Endocrinology* 143, 185–190.
- Levey, A.I., Edmunds, S.M., Heilman, C.J., Desmond, T.J., Frey, K.A., 1994. Localization of muscarinic M3 receptor protein and M3 receptor binding in rat brain. *Neuroscience* 63, 207–221.
- Li, J., Gautam, D., Han, S., Guettier, J., Cui, Y., Lu, H., Deng, C., O'Hare, J., Jou, W., Gavrilova, O., Buettner, C., Wess, J., 2009. Hepatic muscarinic acetylcholine receptors are not critically involved in maintaining glucose homeostasis in mice. *Diabetes* 58, 2776.
- Li, Y., Wu, X., Zhu, J., Yan, J., Owyang, C., 2003. Hypothalamic regulation of pancreatic secretion is mediated by central cholinergic pathways in the rat. *J. Physiol.* 552, 571–587.
- Liddle, R.A., 2000. Regulation of cholecystokinin secretion in humans. *J. Gastroenterol.* 35, 181–187.
- Lieberman, J.A., Stroup, T.S., McEvoy, J.P., Swartz, M.S., Rosenheck, R.A., Perkins, D.O., Keefe, R.S.E., Davis, S.M., Davis, C.E., Lebowitz, B.D., Severe, J., Hsiao, J.K., 2005. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *N. Engl. J. Med.* 353, 1209–1223.
- Matsui-Sakata, A., Ohtani, H., Sawada, Y., 2005. Receptor occupancy-based analysis of the contributions of various receptors to antipsychotics-induced weight gain and diabetes mellitus. *Drug Metab. Pharmacokinet.* 20, 368–378.
- Michel, A.D., Stefanich, E., Whiting, R.L., 1989. Direct labeling of rat M3-muscarinic receptors by [3H]4DAMP. *Eur. J. Pharmacol.* 166, 459–466.
- Newcomer, J., 2005. Second-generation atypical antipsychotics and metabolic effects. A comprehensive literature review. *CNS Drugs* 19, 1–93.
- Ochi, Y., Horie, S., Maruyama, T., Watanabe, K., Yano, S., 2005. Necessity of intracellular cyclic AMP in inducing gastric acid secretion via muscarinic M3 and cholecystokinin2 receptors on parietal cells in isolated mouse stomach. *Life Sci.* 77, 2040–2050.
- Oriot, P., Feys, J., Mertens de Wilmars, S., Misson, S., Ayache, A., Fagnart, O., Gruson, D., Luts, A., Jamart, J., Hermans, M.-P., Buyschaert, M., 2008. Insulin sensitivity, adjusted beta-cell function and adiponectinaemia among lean drug-naïve schizophrenic patients treated with atypical antipsychotic drugs: a nine-month prospective study. *Diab. Metab.* 34, 490–496.
- Paxinos, G., Watson, C., 2007. *The Rat Brain in Stereotaxic Coordinates*, 6th ed. Elsevier Academic Press Inc., CA, USA.
- Perez-Iglesias, R., Vazquez-Barquero, J.L., Amado, J.A., Berja, A., Garcia-Unzueta, M.T., Pelayo-Teran, J.M., Carrasco-Marin, E., Mata, I., Crespo-Facorro, B., 2008. Effect of antipsychotics on peptides involved in energy balance in drug-naïve psychotic patients after 1 year of treatment. *J. Clin. Psychopharmacol.* 28, 289–295.
- Ramankutty, G., 2002. Olanzapine-induced destabilization of diabetes in the absence of weight gain. *Acta Psychiatr. Scand.* 105, 235–236.
- Reagan-Shaw, S., Nihal, M., Ahmad, N., 2007. Dose translation from animal to human studies revisited. *FASEB J.* 22, 659–661.
- Renuka, T.R., Ani, D.V., Paulose, C.S., 2004. Alterations in the muscarinic M1 and M3 receptor gene expression in the brain stem during pancreatic regeneration and insulin secretion in weanling rats. *Life Sci.* 75, 2269–2280.
- Reynolds, G.P., Kirk, S.L., 2010. Metabolic side effects of antipsychotic drug treatment – pharmacological mechanisms. *Pharmacol. Ther.* 125, 169–179.
- Rohner-Jeanrenaud, F., Jeanrenaud, B., 1980. Consequences of ventromedial hypothalamic lesions upon insulin and glucagon secretion by subsequently isolated perfused pancreases in the rat. *J. Clin. Investig.* 65, 902–910.
- Ruiz de Azua, I., Gautam, D., Guettier, J.-M., Wess, J., 2011. Novel insights into the function of [beta]-cell M3 muscarinic

- acetylcholine receptors: therapeutic implications. *Trends Endocrinol. Metab.* 22, 74–80.
- Shen, K.-Z., Johnson, S.W., 2000. Presynaptic dopamine D2 and muscarinic M3 receptors inhibit excitatory and inhibitory transmission to rat subthalamic neurones in vitro. *J. Physiol.* 525, 331–341.
- Silvestre, J., Prous, J., 2005. Research on adverse drug events. I. Muscarinic M3 receptor binding affinity could predict the risk of antipsychotics to induce type 2 diabetes. *Methods Find. Exp. Clin. Pharmacol.* 27, 289–304.
- Smith, G.P., Jerome, C., Cushin, B.J., Eterno, R., Simansky, K.J., 1981. Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. *Science* 213, 1036–1037.
- Starrenburg, F.C.J., Bogers, J.P.A.M., 2009. How can antipsychotics cause diabetes mellitus? Insights based on receptor-binding profiles, humoral factors and transporter proteins. *Eur. Psychiatry* 24, 164–170.
- Tebbe, J.J., Dietze, T., Grote, C., Mönnikes, H., 2001. Excitatory stimulation of neurons in the arcuate nucleus inhibits gastric acid secretion via vagal pathways in anesthetized rats. *Brain Res.* 913, 10–17.
- Terry Jr., A.V., Gearhart, D.A., Mahalik, S.P., Warsi, S., Waller, J.L., 2006. Chronic treatment with first or second generation antipsychotics in rodents: effects on high affinity nicotinic and muscarinic acetylcholine receptors in the brain. *Neuroscience* 140, 1277–1287.
- Toshinai, K., Mondal, M.S., Nakazato, M., Date, Y., Murakami, N., Kojima, M., Kangawa, K., Matsukura, S., 2001. Upregulation of ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochem. Biophys. Res. Commun.* 281, 1220–1225.
- Weston-Green, K., Huang, X.-F., Deng, C., 2011. Olanzapine treatment and metabolic dysfunction: a dose response study in female Sprague Dawley rats. *Behav. Brain Res.* 217, 337–346.
- White, J., Olchovsky, D., Kershaw, M., Berelowitz, M., 1990. Increased hypothalamic content of preproneuropeptide-Y messenger ribonucleic acid in streptozotocin-diabetic rats. *Endocrinology* 126, 765–772.
- Yamada, M., Miyakawa, T., Duttaroy, A., Yamanaka, A., Moriguchi, T., Makita, R., Ogawa, M., Chou, C.J., Xia, B., Crawley, J.N., Felder, C.C., Deng, C.-X., Wess, J., 2001. Mice lacking the M3 muscarinic acetylcholine receptor are hypophagic and lean. *Nature* 410, 207–212.
- Yang, T.-T., Chang, C.-K., Tsao, C.-W., Hsu, Y.-M., Hsu, C.-T., Cheng, J.-T., 2009. Activation of muscarinic M-3 receptor may decrease glucose uptake and lipolysis in adipose tissue of rats. *Neurosci. Lett.* 451, 57–59.
- Zavitsanou, K., Nguyen, V., Han, M., Huang, X., 2007. Effects of typical and atypical antipsychotic drugs on rat brain muscarinic receptors. *Neurochem. Res.* 32, 525–532.
- Zubieta, J.K., Frey, K.A., 1993. Autoradiographic mapping of M3 muscarinic receptors in the rat brain. *J. Pharmacol. Exp. Ther.* 264, 415–422.

CHAPTER FOUR

ALTERATIONS TO MELANOCORTINERGIC, GABAERGIC AND CANNABINOID NEUROTRANSMISSION ASSOCIATED WITH OLANZAPINE-INDUCED WEIGHT GAIN

Reprinted from PLoS One, 7(3): e33548, Weston-Green K, Huang XF and Deng C, Alterations to melanocortinergic, GABAergic and cannabinoid neurotransmission associated with olanzapine-induced weight gain, Copyright (2012) Weston-Green et al. under the Creative Commons Attribution License.

Alterations to Melanocortinergic, GABAergic and Cannabinoid Neurotransmission Associated with Olanzapine-Induced Weight Gain

Katrina Weston-Green^{1,2}, Xu-Feng Huang^{1,2}, Chao Deng^{1,2*}

¹ Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, Wollongong, Australia, ² Schizophrenia Research Institute, Darlinghurst, Australia

Abstract

Background/Aim: Second generation antipsychotics (SGAs) are used to treat schizophrenia but can cause serious metabolic side-effects, such as obesity and diabetes. This study examined the effects of low to high doses of olanzapine on appetite/metabolic regulatory signals in the hypothalamus and brainstem to elucidate the mechanisms underlying olanzapine-induced obesity.

Methodology/Results: Levels of pro-opiomelanocortin (POMC), neuropeptide Y (NPY) and glutamic acid decarboxylase (GAD₆₅, enzyme for GABA synthesis) mRNA expression, and cannabinoid CB1 receptor (CB1R) binding density (using [³H]SR-141716A) were examined in the arcuate nucleus (Arc) and dorsal vagal complex (DVC) of female Sprague Dawley rats following 0.25, 0.5, 1.0 or 2.0 mg/kg olanzapine or vehicle (3×/day, 14-days). Consistent with its weight gain liability, olanzapine significantly decreased anorexigenic POMC and increased orexigenic NPY mRNA expression in a dose-sensitive manner in the Arc. GAD₆₅ mRNA expression increased and CB1R binding density decreased in the Arc and DVC. Alterations to neurotransmission signals in the brain significantly correlated with body weight and adiposity. The minimum dosage threshold required to induce weight gain in the rat was 0.5 mg/kg olanzapine.

Conclusions: Olanzapine-induced weight gain is associated with reduced appetite-inhibiting POMC and increased NPY. This study also supports a role for the CB1R and GABA in the mechanisms underlying weight gain side-effects, possibly by altering POMC transmission. Metabolic dysfunction can be modelled in the female rat using low, clinically-comparable olanzapine doses when administered in-line with the half-life of the drug.

Citation: Weston-Green K, Huang X-F, Deng C (2012) Alterations to Melanocortinergic, GABAergic and Cannabinoid Neurotransmission Associated with Olanzapine-Induced Weight Gain. PLoS ONE 7(3): e33548. doi:10.1371/journal.pone.0033548

Editor: Alice Y. W. Chang, Kaohsiung Chang Gung Memorial Hospital, Taiwan

Received: October 18, 2011; **Accepted:** February 11, 2012; **Published:** March 16, 2012

Copyright: © 2012 Weston-Green et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was supported by a University of Wollongong (www.uow.edu.au) University Research Centre (URC) grant to CD, an Australian National Health and Medical Research Council (NHMRC, www.nhmrc.gov.au) grant to XH and CD (ID 635231), and by the Schizophrenia Research Institute (SRI, www.schizophreniaresearch.org.au), utilising infrastructure funding from New South Wales Health. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have read the journal's policy and have the following conflicts: CD received an honorarium from Eli Lilly, Australia, for presenting at the Cutting Edge Debate, Melbourne (2010). This does not alter the authors' adherence to all PLoS ONE policies on sharing data and materials.

* E-mail: chao@uow.edu.au

Introduction

The second generation antipsychotic (SGA) olanzapine is prescribed to treat schizophrenia and a growing number of other disorders in adults and children [1–8], but can cause adverse metabolic side-effects including increased body weight [9], caloric intake [10,11] and abdominal adiposity [12,13], and reduced physical activity [14–16]. Metabolic side-effects are a growing concern due to co-morbidities such as diabetes and obesity [17], and are a risk factor for medication non-compliance [18]. A number of potential mechanisms for SGA-induced metabolic dysfunction have emerged over the past few years [19–21]. In particular, the histaminergic, serotonergic and dopaminergic neurotransmitter systems are thought to be highly implicated in SGA-induced body weight gain [21–26]. However, SGAs have a broad receptor binding profile that allows direct and indirect effects on multiple neural and peripheral signalling pathways

[26], and further research into other candidate systems is required.

The hypothalamic arcuate nucleus (Arc) and the dorsal vagal complex (DVC) of the brainstem are well-documented for their role in appetite and energy homeostasis [27–29]; responding to the acute nutritional status and long-term regulation of energy stores in the body. Neurons of the Arc and DVC express G_{i/o}-coupled cannabinoid CB1 receptors (CB1R) [30,31], which facilitate the effects of cannabinoids on appetite and energy metabolism [32]. Weight gain during olanzapine and clozapine treatment is associated with a CB1R gene polymorphism in individuals with chronic schizophrenia [33], and chronic high-dose risperidone treatment increases cannabinoid receptor agonist, [³H]CP-55940, binding density in the Arc of male rats [34]. We previously demonstrated a decrease in [³H]CP-55940 binding density in the DVC of rats treated with olanzapine, but not aripiprazole or haloperidol [35]. However, whether changes in receptor density

were attributed to the CB1R is unclear due to the low specificity of the ligand used [36] and localisation of cannabinoid CB2 receptors in the brain [37]. Moreover, the effects of olanzapine on CB1R density in the Arc remain unknown.

The appetite enhancing effects of the major neuronal inhibitor, γ -aminobutyric acid (GABA) in the hypothalamus were reported more than 30 years ago [38]. GABAergic neurons in the Arc are sensitive to leptin [39], and GABA receptor agonists and antagonists stimulate and suppress feeding behaviour, respectively [40]. Down-regulated expression of glutamic acid decarboxylase (GAD, the GABA synthesising enzyme) has been observed in individuals with schizophrenia, bipolar and mood disorder, whereas antipsychotic drug treatment increases cortical GAD expression in rats and primates [41]. GAD exists as two isoforms, 65 and 67; the latter is found throughout the neuronal cytoplasm, whereas GAD₆₅ is located primarily in the axon terminal [42–44] and is the predominant transcript in the hypothalamus of the adult rat brain [45]. However, to our knowledge the effects of olanzapine on GAD₆₅ mRNA expression in the hypothalamic Arc or the DVC have not been investigated.

The Arc and DVC both express mRNA for orexigenic neuropeptide Y (NPY) and anorexigenic pro-opiomelanocortin (POMC) [46–48]. The POMC gene encodes for neuropeptides such as adrenocorticotrophic hormone, β -endorphin and α -melanocortin stimulating hormone; the latter of which exerts its anorexigenic effects largely through melanocortin-3 and melanocortin-4 receptor (MC4-R) subtypes [49]. Conversely, the central application of NPY induces food intake in a number of species [50], hypolocomotor activity in rats [51,52], and can lead to obesity following chronic over-exposure [53,54]. Therefore, it is possible that interference in the balance of POMC and NPY by olanzapine may contribute to the drug's obesogenic liability. Several reports demonstrated increased NPY immunoreactivity in the Arc of clozapine-treated rats [55,56], whereas chronic risperidone treatment in male rats had no effect on POMC or NPY expression, or body weight [34], which may be due to the lower sensitivity of male rats to SGA-induced metabolic side-effects compared to females [57–60]. Other studies have examined antipsychotic effects on NPY mRNA expression in the brain with region-dependent outcomes [61–65]. The effects of antipsychotics on POMC or NPY in the brainstem have not been examined and studies on hypothalamic appetite-regulating peptides during olanzapine treatment are confounding; one group reported an increase in orexigenic NPY and AgRP and a concurrent reduction in appetite-inhibiting POMC and cocaine- and amphetamine-related transcript (CART) [66], whilst another reported no change in several hypothalamic peptides, including NPY and POMC [67]. A key factor that may contribute to the difference in findings is drug dosage (i.e.: 1 mg/kg olanzapine [66] *vs.* a supratherapeutic dose of 3 mg/kg olanzapine [67] (b.i.d.). Indeed, metabolic outcomes can differ with antipsychotic dosage [68–71] and increased dose induces greater metabolic dysfunction in the rat [66,72,73], however, high antipsychotic dosages in the rat may not represent the clinic [74]. In addition, both studies had a large dosage interval, i.e.: 6–7 then 17–18 hourly treatments, b.i.d. [66,67]. As the half-life of olanzapine is 5.1 hours in the rat brain with high levels remaining after 8-hours [75], compared to approximately 75.2 hours in the human brain [76], multiple dosages are required in the rat in order to minimise drug fluctuations below sub-therapeutic D2 receptor occupancy levels [74,77]. Therefore, it may be possible to model olanzapine-induced metabolic dysfunction in the rat using low olanzapine dosages when administered in accordance with the half-life of the drug, i.e.: 8 hourly (t.i.d.) within 24-hours.

Using an established rat model of olanzapine-induced metabolic dysfunction [35,70,78–80], this study aimed to investigate the mechanisms underlying weight gain associated with olanzapine treatment by examining its effects on POMC, NPY and GAD₆₅ mRNA expression, and CB1R binding density (using the CB1R-specific ligand [³H]-SR141716A) in the Arc and DVC. Statistical correlations between these parameters in the brain and body weight, food intake and visceral adiposity were investigated. To identify the minimum dosage threshold required to induce metabolic change, rats were treated with different clinically-relevant olanzapine dosages, calculated based on comparable therapeutic *in-vivo* dopamine D2 receptor occupancy levels [74] and differences in body surface area between species [81]. Collectively, the present study demonstrates that olanzapine changes the balance of anorexigenic POMC and orexigenic NPY mRNA expression in the Arc, does not alter POMC or NPY in the DVC, and increases GAD₆₅ mRNA expression but reduces CB1R density in both the hypothalamus and brainstem. These largely dose-sensitive changes may underlie a shift in energy balance that favours weight gain during olanzapine treatment. Metabolic dysfunction can be modelled in the female rat using low olanzapine doses when administered in-line with the half-life of the drug.

Methods

Ethics Statement

All experimental procedures were approved by the Animal Ethics Committee (Approval #: AE06/32), University of Wollongong, and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004). All efforts were made to minimise animal distress and prevent suffering.

Animals and Treatment

Seven week-old female Sprague Dawley rats (Animal Resources Centre, Perth, WA, Australia), housed in 12-h light dark cycle (lights on 07:00, 22°C) were habituated for 1-week, then randomly assigned to 0.25, 0.5, 1.0 or 2.0 mg olanzapine/kg (Zyprexa, Eli Lilly, Indianapolis, IN, USA) or vehicle (control) (n = 12), administered three-times daily in a sweet cookie dough pellet, as described previously [70]. Briefly, olanzapine tablets were de-coated and pulverized then the assigned dosage was added to measured dry ingredients. Water droplets were added immediately prior to administration to achieve a dry-dough consistency. After a 1-week teaching period, rats learnt to voluntarily self-administer a 0.3 g cookie-dough pellet either containing the assigned dosage of olanzapine, or plain cookie-dough without the drug (control group), offered by a metal spoon at 8-hourly intervals (3 pellets/day) for 14-days. Consumption of each pellet was observed to ensure complete dosing. Body weight and food intake measurements were recorded (n = 12). Animals were allowed *ad libitum* access to water and standard laboratory chow diet throughout the study. Animals were fasted for 4–6 hours then euthanized using sodium pentobarbitone 10–12 hours after the last treatment. Brain tissue was immediately frozen in liquid nitrogen and stored at –80°C. Visceral (perirenal and periovary) white fat pads were dissected and weighed (n = 12). Six brains were randomly selected from each treatment group for use in the mRNA expression and receptor binding experiments. Tissue was sectioned (14 μ m, –18°C) along the coronal plane then stored at –20°C.

NPY, POMC and GAD₆₅ mRNA In-Situ Hybridisation

POMC mRNA expression was observed using in-situ hybridisation techniques previously described by our laboratory [82],

using the following specific antisense hybridisation probe: 5'-CGTTCTTGATGATGGCGTTCTTGAAGAGCGTCACCAAGGGCGTCT-3' (J00612, 547–591). NPY mRNA expression was observed using in-situ hybridisation techniques previously described by our laboratory [65,82], using the following specific antisense hybridisation probe: 5'-GAGTGTATCTGGCCATGTCCTCTGCTGGCGCGTCCCTCGCCCGG-3' (M15792, 1650–1693). GAD₆₅ mRNA expression was observed using the following specific antisense hybridisation probe: 5'-GGCGTCCACACTGCAAGGCCTTGTCTCCCGTGTTCATAGGACAGGTCAT-3' (NM_012563.1, 1419–1372), as previously described by Ling et al., [83]. Oligonucleotide probes were terminally labelled using [³²S]dATP (1000 Ci/mmol, Perkin Elmer, Waltham, MA, USA) in 10-fold molar excess and terminal transferase (Promega, Madison, WI, USA), then purified using a MicroSpin G-50 column (GE Healthcare Ltd, Buckinghamshire, UK). Hybridisation was performed by incubating slides in hybridisation buffer (4× SSC, 1× Denhardt's solution, 50% de-ionised formamide, 200 µg/ml sperm DNA, 100 µg/1 ml polyA, 120 µg/ml heparin, 20 mM sodium phosphate and labelled probe, pH 7.0) for 18-hours at 37°C. Slides were then washed in 1× SSC buffer at 55°C (3×30-minutes each) and incubated for 1-hour in SSC buffer at room temperature. Sections were dipped in Milli-Q water followed sequentially by 70% then 95% ethanol, and dried under a gentle stream of air. Autoradiographic images were captured on film (Kodak BioMax MR film, Rochester, NY, USA) exposed for 3-weeks. Films were quantified using a GS-800 Densitometer (Bio-Rad Laboratories, Inc), and analysis software (Quantity One, v4.6.7, Bio-Rad Laboratories, Inc, CA, USA). Values were derived from a standard curve generated from a [¹⁴C]-labelled autoradiographic standard (GE Healthcare Ltd, Buckinghamshire, UK) (mean binding nCi/g tissue equivalent vs. density). Slides were dipped in Emulsion solution (GE Healthcare Ltd, Buckinghamshire, UK) and exposed for 6-weeks, then stained with cresyl violet (Nissl stain) (Sigma-Aldrich, NSW, Australia), to allow further examination of positive signals at the cellular level.

CB1R Binding Density

CB1R binding density was detected using methods previously published by our laboratory [84]. Briefly, air-dried slides were pre-incubated for 15 min in incubation buffer containing 50 mM Tris HCl buffer (pH 7.4) and 0.1% bovine serum albumin, at room temperature. Sections were then incubated with 10 nM [³H] SR141716A (52 Ci/mMol, Amersham, UK), a CB1R-specific inverse agonist, in buffer (pH 7.4) at room temperature for

60 minutes to determine total binding. Non-specific binding was determined by incubating subsequent sections in 10 nM [³H] SR141716A in the presence of 100 µM CP-55940, in buffer (pH 7.4) for 60 minutes at room temperature. Slides were washed in ice cold buffer (pH 7.4), (2×30 minutes), then dipped in distilled water and dried under a gentle stream of cool air. CB1R autoradiographic images were captured using a Beta Image camera (BioSpace, Paris, France), which counts the amount of β-particles emitted from the tissue (3.5 hours exposure) to determine the level of radioactivity bound to the brain sections. Radioactive levels were obtained in counts per minute per square millimetre of tissue (cpm/mm²), converted to nCi/mg tissue equivalent using standard tissue sections calibrated with commercial standards (Amersham, Buckinghamshire, United Kingdom), then transformed into fmol/mg tissue equivalent by taking into account the specific activity of the radioligand (52 Ci/mMol). Quantification was conducted using β-Image Plus software (version 4, BioSpace, Paris, France).

Quantification and Statistical Analysis

Quantification of autoradiographic images was performed on the hypothalamic Arc and the DVC of the brainstem, which were confirmed using a corresponding set of cresyl violet-stained slides and a standard rat brain atlas [85]. Data were analysed using SPSS (version 17.0, SPSS, Chicago, IL, USA). All data points were within ±2 standard deviations. One-Sample Kolmogorov-Smirnov tests revealed normal data distribution. One-way ANOVAs were employed to determine the effect of treatment on percentage body weight change, food intake, visceral adiposity, as well as NPY, POMC and GAD₆₅ mRNA expression, and CB1R binding density in the hypothalamus and brainstem. ANOVAs were followed by multiple comparisons using post-hoc Dunnett-T tests where relevant ($p < 0.05$). Correlations were identified using Pearson's correlation tests.

Results

Body Weight, Food Intake and Visceral Adiposity

There was a significant effect of treatment on the percentage of body weight change from treatment day 0 ($F_{4,55} = 7.68$, $p < 0.01$). Compared to controls, olanzapine significantly increased percentage of body weight change in the 0.5 mg/kg ($p < 0.05$), 1.0 mg/kg and 2.0 mg/kg ($p < 0.01$) treatment groups, but not in the low dosage group of 0.25 mg/kg ($p > 0.05$) (Figure 1A). Mean cumulative food intake significantly increased in the 2.0 mg/kg

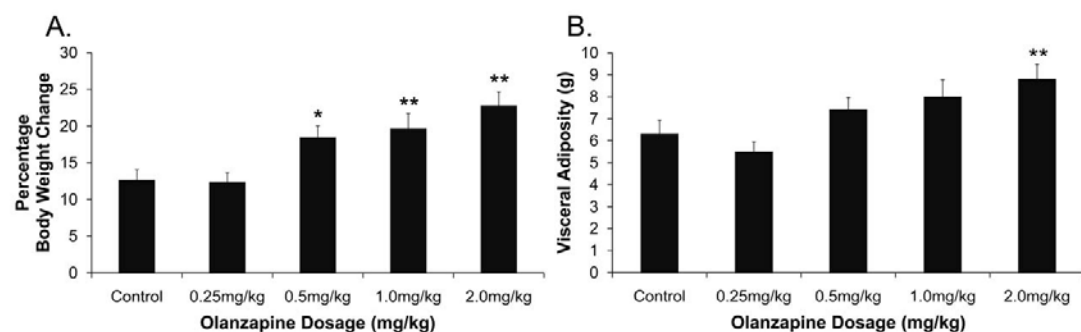


Figure 1. Weight Gain and Visceral Adiposity. (A): Percentage body weight change and (B): visceral adiposity in female Sprague Dawley rats ($n = 12$ /treatment group) treated with 0.25, 0.5, 1.0 or 2.0 mg/kg olanzapine or vehicle (control), (14-days, t.i.d). Data expressed as mean ± SEM. * $p < 0.05$, ** $p < 0.01$ vs. control.

doi:10.1371/journal.pone.0033548.g001

olanzapine dosage group compared to the control group (318.6 ± 11.2 g *vs.* 269.4 ± 11.1 g, $p < 0.05$). An increase (7.8%) in food intake was also observed in the 0.5 mg/kg and 1.0 mg/kg olanzapine treatment groups, but did not reach significance compared to the controls (0.5 mg/kg: 289.1 ± 13.9 g *vs.* 269.4 ± 11.1 g, 1.0 mg/kg: 291.6 ± 12.1 g *vs.* 269.4 ± 11.1 g, $p > 0.05$), and the low dosage group (0.25 mg/kg) did not differ to the control group (265.4 ± 11.7 g *vs.* 269.4 ± 11.1 g). Olanzapine treatment had a significant effect on visceral adiposity ($F_{4,55} = 4.60$, $p < 0.01$), with a significant increase observed in the 2.0 mg/kg olanzapine treatment group ($p < 0.05$) and a trend for an increase in the 1.0 mg/kg dosage group ($p = 0.09$), but not in the lower dosage groups ($p > 0.05$) (Figure 1B).

POMC and NPY mRNA Expression

Examples of POMC and NPY mRNA expression in the hypothalamus are shown in Figure 2A–D. Olanzapine had a significant effect on POMC mRNA expression in the Arc ($F_{4,25} = 8.32$, $p < 0.01$), not in the DVC ($F_{4,25} = 1.44$, $p = 0.25$) (Figure 3A). Post-hoc analysis identified a significant decrease in POMC mRNA expression in the Arc following dosages of 0.5 mg/kg, 1.0 mg/kg and 2.0 mg/kg ($p < 0.01$) olanzapine, but not 0.25 mg/kg olanzapine ($p = 0.90$), compared to controls (Figure 3A). There was also a significant effect of treatment on NPY mRNA expression in the Arc ($F_{4,25} = 8.55$, $p < 0.01$), with a significant increase in the Arc following 1.0 mg/kg and 2.0 mg/kg olanzapine (Figure 3B), but not in the lower dosage groups ($p = 0.34$ and $p = 0.11$ for 0.25 mg/kg and 0.5 mg/kg olanzapine, respectively) (Figure 3B). NPY mRNA expression in the DVC was unaltered by olanzapine ($F_{4,25} = 0.73$, $p = 0.58$) (Figure 3B).

GAD₆₅ mRNA Expression

Examples of GAD₆₅ mRNA expression are shown in Figure 2E–F. A significant effect of treatment on GAD₆₅ mRNA expression was observed in the Arc ($F_{4,25} = 5.21$, $p < 0.01$) and DVC ($F_{4,25} = 7.73$, $p < 0.01$), with an increase following olanzapine dosages of 1.0 mg/kg (Arc $p < 0.05$, DVC $p < 0.01$) and 2.0 mg/kg (both regions $p < 0.01$), but not in the 0.5 mg/kg or 0.25 mg/kg groups ($p > 0.05$), compared to controls (Figure 3C).

CB1R Binding Density

An example of CB1R binding density in the hypothalamus and DVC is shown in Figure 4. There was a significant effect of treatment on CB1R binding density in the Arc ($F_{4,25} = 7.48$, $p < 0.01$), with a reduction in all olanzapine treatment groups compared to controls ($p < 0.01$) (Figure 3D). Olanzapine decreased CB1R binding density in the DVC ($F_{4,25} = 3.48$, $p < 0.05$) of animals treated with 0.5 mg/kg, 1.0 mg/kg or 2.0 mg/kg olanzapine ($p < 0.05$), but not 0.25 mg/kg olanzapine ($p = 0.43$) (Figure 3D).

Correlations

POMC mRNA expression in the Arc significantly correlated to percentage body weight change ($r = -0.43$, $p < 0.05$), visceral adiposity ($r = -0.49$, $p < 0.01$), NPY mRNA expression ($r = -0.45$, $p < 0.05$), and GAD₆₅ mRNA expression in the Arc ($r = -0.54$, $p < 0.01$) (Figure 5A–D). There was a significant positive correlation between NPY and GAD₆₅ mRNA expression in the Arc ($r = 0.69$, $p < 0.01$) (Figure 5E), however the two factors did not correlate to percentage body weight change ($p > 0.05$). CB1R binding density in the DVC correlated to percentage body weight change ($r = -0.38$, $p < 0.05$), visceral adiposity ($r = -0.38$, $p < 0.05$) and GAD₆₅ mRNA expression in the DVC ($r = -0.52$, $p < 0.01$)

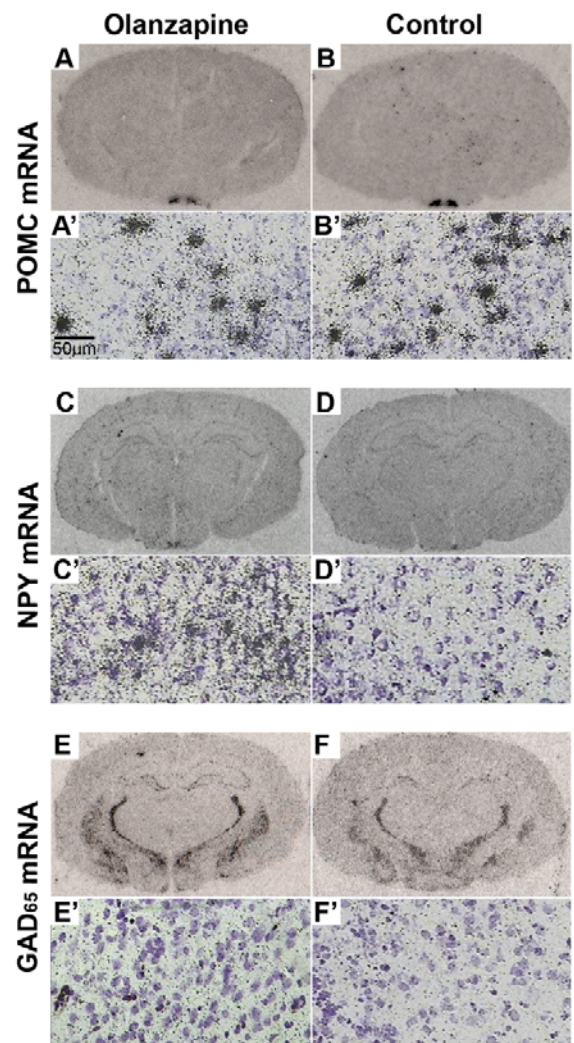


Figure 2. Examples of POMC, NPY and GAD₆₅ mRNA Expression Following Olanzapine Treatment. Examples of pro-opiomelanocortin (POMC), neuropeptide Y (NPY) and glutamic acid decarboxylase 65 (GAD₆₅) mRNA expression in the female Sprague Dawley rat brain following 2.0 mg/kg olanzapine treatment (A–A', C–C', E–E') or vehicle (control) (B–B', D–D', F–F') for 14-days (t.i.d.). (A–F): Low magnification film autoradiographs depicting mRNA expression in the rat brain, (A'–F'): High magnification emulsion/cresyl violet-stained slides showing mRNA expression specifically in the arcuate nucleus. Autoradiographs are examples of raw data used for the graphs depicted in Figure 3A–C and are average representations of 6 rats per treatment group.

doi:10.1371/journal.pone.0033548.g002

(Figure 5F–H), and a weak correlation was observed between CB1R binding density in the Arc and percentage body weight change ($r = -0.33$, $p = 0.08$).

Discussion

We found that olanzapine alters signals in the hypothalamus and brainstem that are implicated in appetite and energy

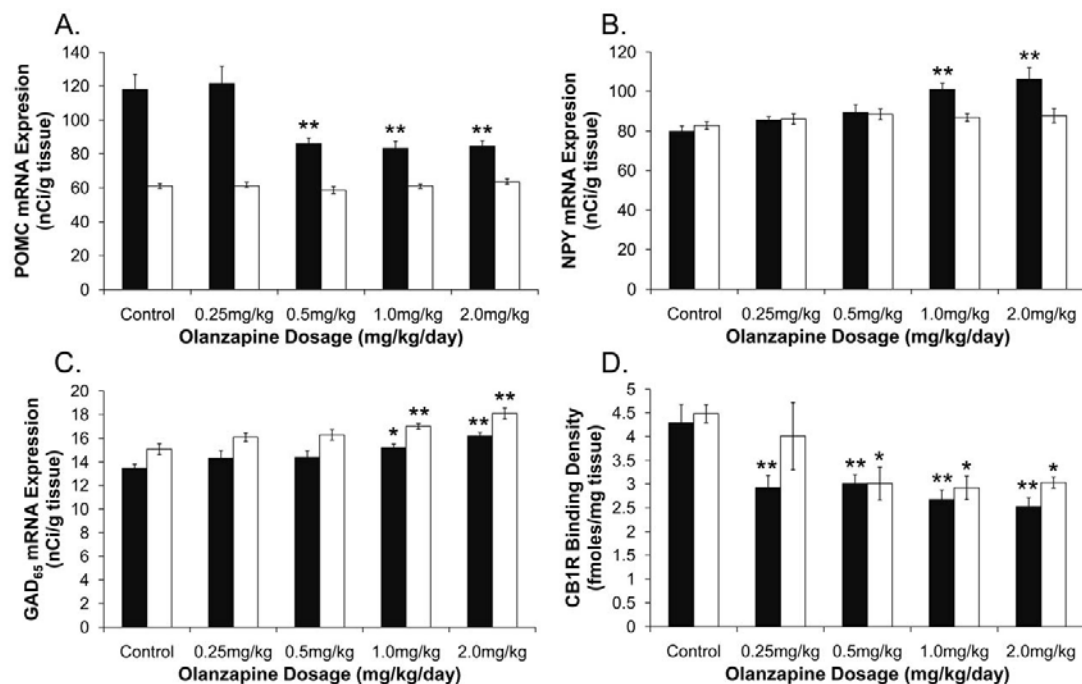


Figure 3. Dosage Effects of Olanzapine Treatment on POMC, NPY and GAD₆₅ mRNA Expression, and CB1R Binding Density. (A): Proopiomelanocortin mRNA expression (nCi/g tissue), (B): neuropeptide Y mRNA expression (nCi/g tissue), (C): glutamic acid decarboxylase (GAD₆₅) mRNA expression (nCi/g tissue), (D): cannabinoid CB1 receptor binding density (fmol/mg tissue), in rats treated with 0.25, 0.5, 1.0 or 2.0 mg/kg olanzapine or vehicle (control) (14-days, t.i.d.) ($n = 6$ /treatment group). Key: ■ arcuate nucleus □ dorsal vagal complex. Data is expressed as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$ vs. control. doi:10.1371/journal.pone.0033548.g003

homeostasis in a largely dose-sensitive manner. These changes may underlie a shift in energy balance that favours weight gain. The data support a role for POMC in the mechanisms underlying

olanzapine-induced obesity. Reduced POMC satiety signalling leads to obesity in the clinic and in animal models of obesity, for example, POMC mRNA expression is attenuated in genetically obese Zucker rats [86], tubby mice (*tubby* gene mutation) [87] and diet-induced obese mice [88]. In addition, genetic POMC deficiency leads to obesity in humans [89] and mice [90], and MC4-R deficiency leads to morbid obesity associated with enhanced adiposity and chronic hyperphagia [91]. The result of unaltered POMC mRNA expression in the DVC was not entirely surprising as the role of POMC neurons in the DVC is not well-characterised, and functional and chemical distinctions to the Arc have been identified [92,93].

NPY mRNA expression was upregulated in the Arc following 1.0 mg/kg and 2.0 mg/kg olanzapine treatment, however no significant correlation with weight gain was observed. This is consistent with some NPY transgenic and deficiency models i.e.: mice and rats that over-express NPY do not have a hyperphagic/obese phenotype [94], and genetic modelling of NPY-deficiency does not result in reduced body weight, adiposity, or food intake [95–97]. However, it is possible that NPY had an indirect effect on weight gain in olanzapine-treated animals, for example by inhibiting POMC. Indeed, NPY neurons synapse on POMC cell bodies and can inhibit their spontaneous activity [98–100], however, unlike POMC, NPY mRNA expression did not change in the 0.5 mg/kg olanzapine treatment group suggesting a role for other systems in POMC regulation. The dosage response of NPY mRNA expression was in-line with the increase in GAD₆₅ mRNA expression in the 1.0 mg/kg and 2.0 mg/kg olanzapine treatment groups, although GAD₆₅ mRNA expression increased in both the

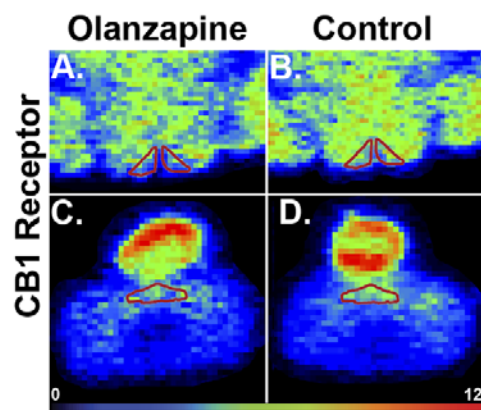


Figure 4. Example of CB1R Binding Density Following Olanzapine Treatment. Example of cannabinoid CB1 receptor binding density (using [³H]SR-141716A) in the A, C: hypothalamic arcuate nucleus and B, D: dorsal vagal complex of the caudal brainstem of female Sprague Dawley rats following A, B: 2.0 mg/kg olanzapine treatment, C, D: vehicle (control) for 14-days (t.i.d.). Autoradiographs are examples of raw data used for the graphs depicted in Figure 3D and are average representations of 6 rats per treatment group. doi:10.1371/journal.pone.0033548.g004

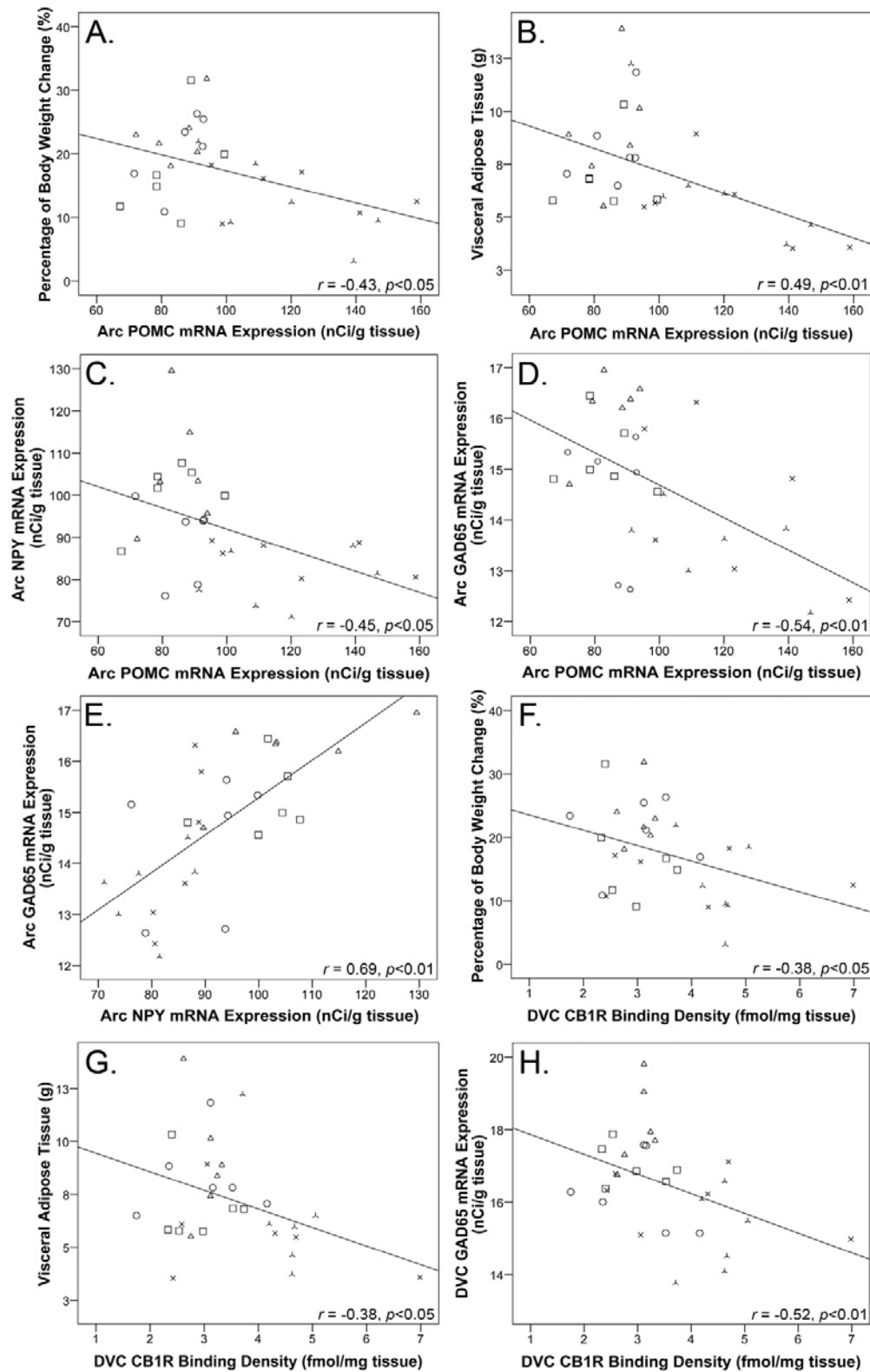


Figure 5. Correlations. Correlations between pro-opiomelanocortin (POMC) mRNA expression in the arcuate (Arc) nucleus and (A): percentage of body weight change, (B): visceral adipose tissue, (C): neuropeptide Y (NPY) mRNA expression in the Arc, (D): glutamic acid decarboxylase (GAD₆₅) mRNA expression in the Arc, (E): Arc NPY and GAD₆₅ mRNA expression, (F): cannabinoid CB1 receptor (CB1R) binding density in the dorsal vagal complex (DVC) and percentage of body weight change, (G): DVC CB1R binding density and visceral adipose tissue, and (H): DVC CB1R binding density and GAD₆₅ mRNA expression, following 14-days olanzapine treatment or vehicle (control). Correlation analyses were made from raw data underlying the graphs presented in Figures 1 and 3. Key: Δ control, $\times 0.5$ mg/kg, \circ 0.5 mg/kg, \square 1.0 mg/kg, \triangle 2.0 mg/kg olanzapine. doi:10.1371/journal.pone.0033548.g005

Arc and DVC. Upregulated GABAergic signalling during weight gain is consistent with previous reports that whilst NPY and/or AgRP gene deficiency is insufficient to reduce food intake [101], ablation of NPY/AgRP/GABA neurons results in acute hypophagia [102] and deletion of vesicular GABA transporter in AgRP neurons (which co-express NPY) results in a lean, obesity-resistant phenotype in mice [103]. GABA is co-localised in approximately 30% of POMC [104] and NPY/AgRP neurons in the Arc [105], and GABA derived from NPY/GABA axons can suppress spontaneous firing of POMC neurons [98]. In addition, a dense population of leptin-responsive, largely non-AgRP GABAergic neurons that increase inhibitory post-synaptic currents in POMC neurons were recently identified in the Arc [39]. Therefore, the increase in Arc GAD mRNA expression observed in the present study may have arisen from a number of GABAergic sources.

Olanzapine treatment elicited a robust reduction in CB1R density in the Arc and DVC. Using the CB1R-specific ligand, [3 H]-SR141716A, we confirm that our original findings of a reduction in [3 H]CP-55940 binding density in the DVC during olanzapine treatment [35] were attributed to the CB1R sub-type and extend these findings into the hypothalamus. CB1R number and cell signal transduction pathways decrease following over-exposure to agonists [106] and animal models of obesity, for example obese *db/db* and *ob/ob* mice, and fatty Zucker rats, exhibit elevated hypothalamic endocannabinoid levels [107]. Therefore, reduced CB1R binding density following olanzapine treatment may be a result of increased endogenous cannabinoids. Endogenous cannabinoids play an important regulatory role in synaptic transmission by modulating neuronal excitatory and inhibitory input [108,109]. Interestingly, POMC neurons secrete endocannabinoids under basal conditions that retrogradely activate CB1Rs expressed on GABAergic neurons [110,111]. G-protein sub-units coupled to the CB1R inhibit the opening of calcium channels, which reduces vesicular release of GABA [109]. CB1R activation can relieve inhibitory input to the post-synaptic POMC neuron [110–112]. We suggest that reduced CB1R density during olanzapine treatment may diminish cannabinoid-regulated inhibition of GABA, and therefore enhance GABAergic input to POMC neurons, suppressing POMC and encouraging body weight gain (Figure 6). In addition, anandamide and CP-55940 increase NPY release in the hypothalamus [113], therefore, increased endocannabinoid levels may contribute to an increase in NPY during olanzapine treatment that further suppresses POMC (Figure 6). CB1Rs can also modulate GABA and glutamate release in the DVC [30], however the functional implications of changes in CB1R density and GAD₆₅ mRNA expression during olanzapine treatment require further investigation. Additionally, the influence of olanzapine on other neurotransmitter systems may play a role in the mechanisms underlying SGA-induced weight gain [25,114]. For example, olanzapine is a potent histamine H1 receptor antagonist [115] and antipsychotic affinity for the H1 receptor can predict its weight gain liability [114], however the underlying mechanisms for the effect of the H1 receptor on antipsychotic-induced body weight may be independent of melanocortinergic neurotransmission [116]. On the other hand, dopamine D1 and D2 receptor antagonism influences

hypothalamic NPY mRNA expression [117–119] and serotonin 5-HT_{2C} receptor agonists can activate POMC neurons [120,121], therefore, the antagonistic affinity of olanzapine to D2 and 5-HT_{2C} receptors [122,123] may contribute to its weight gain side-effects [24]. These receptors may form broader components of the mechanism proposed in the present study, however further research is necessary.

Our finding of a decrease in POMC and increase in NPY mRNA expression during olanzapine treatment coincide with Ferno et al [66], but contrast to the lack of change reported by Davoodi et al [67]. As discussed earlier, these studies differ in olanzapine dosage and treatment interval [66,67]. Additionally, in Davoodi's study animals were not fasted and PCR methods were used to detect expressional changes in the whole hypothalamus [67], whereas rats were fasted prior to euthanasia and *in-situ* hybridisation techniques were utilised to target expression specifically in the Arc in the present study and [66]. Furthermore, patterns of daily changes in hypothalamic NPY and POMC gene expression have been reported [124], therefore timing of euthanasia may also confound results. A previous study from our laboratory reported a drug withdrawal response of NPY mRNA expression to olanzapine treatment cessation, i.e.: no change in Arc NPY mRNA expression after 2-hour drug washout and a decrease after 48-hour withdrawal after 5-weeks olanzapine treatment [65]. As body weight associated with olanzapine treatment follows a 'peak-and-plateau' trend over time [71,79,125], the lack of change in NPY mRNA expression [65] may be related to compensatory mechanisms that coincide with a plateau in body weight. Further investigation into the time-dependent pattern of NPY mRNA expression during chronic olanzapine treatment would be useful.

Secher et al. [34] reported increased [3 H]CP-55940 binding density in the Arc following 28-days risperidone treatment, and observed a significant correlation between plasma drug levels and visceral adiposity. These results are similar to our study as olanzapine influenced CB1R density in the Arc and changes in CB1R density correlated with adiposity. Differences in the direction of CB1R density change may be attributed to several differences in experimental design in Secher et al.'s study [34], including drug dosage above the upper clinical limit [34], administration method i.e.: continuous drug application via mini-pump with no drug washout period, and treatment duration as time-dependent changes in CB1R density and transduction pathways have been reported [106]. Neither drugs have an affinity for the CB1R ($>10,000$ K_i (nM) [126,127]), indicating that effects on the CB1R are secondary changes and exactly how these SGAs influence CB1Rs should be investigated in future studies. An olanzapine-induced decrease in CB1R binding density seems contrary to the orexigenic influence of CB1R activation, and appetite suppression of CB1R blockade [128]. However, there is vast potential for the endogenous cannabinoid system to modulate metabolism, including central and peripheral effects on food intake and reward aspects of feeding, glucose and lipid metabolism, and energy expenditure [129–136]; aspects of which may contribute to the weight loss efficacy of rimobant [135]. Olanzapine-induced weight gain is associated with increased GABA and decreased

A Proposed Mechanism of Olanzapine-Induced Body Weight Gain

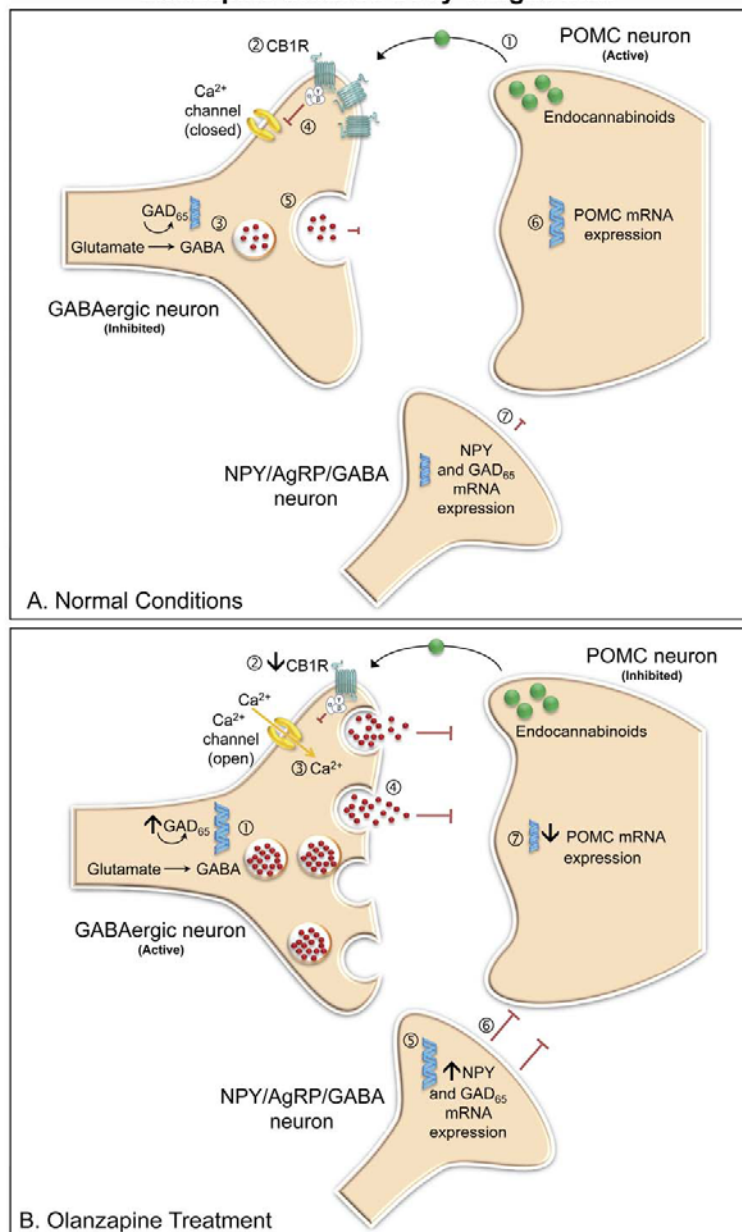


Figure 6. A Proposed Mechanism for Olanzapine-Induced Weight Gain through Interactions Between POMC, NPY, CB1 and GABA systems. (A) **Normal Conditions.** Schematic illustrating proposed inhibitory synaptic transmission to the POMC neuron modulated by NPY, cannabinoid and GABAergic systems under normal conditions. (1) Endogenous cannabinoids are released from the post-synaptic POMC neuron and retrogradely activate CB1 receptors located on the non-AgRP GABAergic neuron (2). GABA is synthesised from glutamate via the rate limiting enzyme GAD (3), however G-protein sub-units coupled to the CB1R inhibit the opening of calcium channels (4), which reduces vesicular release of GABA from the presynaptic terminal to the POMC neuron (5), disinhibiting POMC (6). A number of NPY neurons co-express GABA and can also inhibit POMC. These NPY/AgRP/GABA neurons synapse on POMC neurons and can regulate POMC cell activity (7). (B) **Olanzapine Treatment:** Our data demonstrates that olanzapine decreases POMC mRNA expression and CB1R binding density, whilst simultaneously increasing NPY and GAD mRNA expression. Based on these findings we suggest a potential mechanism contributing to weight gain during olanzapine treatment. (1) Increased GAD mRNA expression enhances the potential for GABA production, whilst (2) decreased CB1R density following olanzapine treatment may remove inhibition of calcium channels (3) and allow vesicular release of GABA (4). The combined effect may be to increase GABA production and release. (5) Olanzapine increases NPY mRNA expression, which can inhibit POMC activation (6). Therefore, reduced CB1R density, and enhanced GAD and NPY

may contribute to the suppression of POMC (7). As POMC is an important anorexigenic peptide, its prolonged inhibition during olanzapine treatment may lead to increased body weight and adiposity side-effects.
doi:10.1371/journal.pone.0033548.g006

CB1R density, whereas anorexigenic rimonabant, decreases GABA release from NPY/AgRP/GABA neurons, possibly via modulation of cannabinoid-sensitive opioid peptides [112]. This suggests that although olanzapine and rimonabant influence the CB1R, they exert their effects through different modes of action.

The doses used in the present study were selected based on the recommended clinical olanzapine dosage range of 5–20 mg/day [70,74,81] excluding the 0.25 mg/kg treatment group, which was included as a minimum response threshold. Olanzapine was administered every 8-hours, based on the half-life of olanzapine in the rat brain [75], to minimise inappropriate peaks and troughs in drug levels between treatments [74]. The present study demonstrates that olanzapine-induced metabolic dysfunction can be modelled in the female rat using low olanzapine dosages when treatment is administered in accordance with the half-life of the drug. In addition, treatment was voluntarily self-administered orally in a cookie-dough pellet, which aimed to minimise potential handling stress [137] and maintain a consistently high drug dosage in the brain [74,75]. Oral drug administration in rats requires a teaching period to ensure voluntary pellet consumption, however this method resembles clinical administration and may circumvent limitations reported using other administration techniques, such as mini-pump, injection and gavage [74,138–140]. Consistent with the clinic [141], olanzapine has a sedative effect in the rat at high doses [72] and we previously reported a general trend of reduced locomotor activity in response to increasing olanzapine dosage [70]. It is possible that sedation plays a role in weight gain during olanzapine treatment, however, as hyperphagia was only apparent in the high dosage group (2 mg/kg olanzapine) in the present study, it is unlikely that sedation influenced the animal's ability to consume food.

In conclusion, our data demonstrates that olanzapine, an antipsychotic drug with a high metabolic liability, alters key metabolic signals in the hypothalamus and brainstem in a manner that favours positive energy balance and may contribute to its weight gain/obesity side-effects. Olanzapine decreases anorexigenic POMC, increases orexigenic NPY, and alters CB1R and

GABAergic signalling in a largely dose-sensitive manner. Low dosages of 0.5 mg/kg and 1.0 mg/kg olanzapine (t.i.d.) were sufficient to induce metabolic changes. Drug dosage may contribute, in-part, to inconsistencies observed between reports in the literature. Enhanced body weight and visceral adiposity during olanzapine treatment are associated with reduced anorexigenic POMC mRNA expression. We propose that increased NPY and enhanced inhibitory GABAergic input, possibly through reduced CB1R density, may contribute to POMC inhibition (Figure 6). However, the present study has several limitations, firstly, statistical correlations do not provide direct evidence of a causal link, and secondly, changes to mRNA and receptor binding density may not reflect a functional protein change, therefore further studies are required to confirm the mechanism proposed in the present study. Examination of olanzapine's effects on the GAD67 isoform and other hypothalamic neuropeptides, such as AgRP and CART, would be useful, as well as investigation into the time-response of all parameters at different intervals during treatment. Finally, as CB1R density decreased in all olanzapine dosage groups, experiments using lower dosages are required to identify the minimum dosage threshold. Taken together, this study supports a role for the melanocortinergic, GABAergic and cannabinoid systems in the underlying mechanisms contributing to olanzapine-induced weight gain side-effects and provides direction on dosage consideration for future animal modelling studies.

Acknowledgments

We sincerely thank Dr Kai Kang, Dr Mei Han, Ms Kelly Liu and Ms Jiamei Lian for their expert technical assistance.

Author Contributions

Conceived and designed the experiments: KWG XH CD. Performed the experiments: KWG. Analyzed the data: KWG XH CD. Contributed reagents/materials/analysis tools: KWG XH CD. Wrote the paper: KWG XH CD.

References

- Ballard C, Waite J, Birks J (2006) Atypical antipsychotics for aggression and psychosis in Alzheimer's disease. *Cochrane Database of Systematic Reviews* 2006: Art. No.: CD003476. DOI: 003410.001002/14651858.CD14003476. pub14651852.
- Brambilla F, Monteleone P, Maj M (2007) Olanzapine-induced weight gain in anorexia nervosa: Involvement of leptin and ghrelin secretion? *Psychoneuroendocrinology* 32: 402–406.
- Bridle C, Palmer S, Bagnall AM, Darba J, Duffy S, et al. (2004) A rapid and systematic review and economic evaluation of the clinical and cost-effectiveness of newer drugs for treatment of mania associated with bipolar affective disorder. *Health Technol Assess* 8: iii–iv.
- Budman G, Gayer A, Lesser M, Shi Q, Bruun R (2001) An open-label study of the treatment efficacy of olanzapine for Tourette's disorder. *J Clin Psychiatry* 62: 290–294.
- Centorrino F, Eakin M, Bahk W-M, Kelleher JP, Goren J, et al. (2002) Inpatient Antipsychotic Drug Use in 1998, 1993, and 1989. *Am J Psychiatry* 159: 1932–1935.
- De Hert M, Dobbelaere M, Sheridan EM, Cohen D, Correll CU (2011) Metabolic and endocrine adverse effects of second-generation antipsychotics in children and adolescents: A systematic review of randomized, placebo controlled trials and guidelines for clinical practice. *Eur Psychiatr* 26: 144–158.
- Frenchman IB (2005) Atypical antipsychotics for nursing home patients: a retrospective chart review. *Drugs & Aging* 22: 257–264.
- Pickar D, Vinik J, Bartko JJ (2006) Pharmacotherapy of Schizophrenic Patients: Preponderance of Off-Label Drug Use. *PLoS ONE* 3: e3150.
- Newcomer J (2005) Second-Generation Atypical Antipsychotics and Metabolic Effects. A Comprehensive Literature Review. *CNS Drugs* 19: 1–93.
- Eder U, Mangweth B, Ebenbichler C, Weiss E, Hofer A, et al. (2001) Association of olanzapine-induced weight gain with an increase in body fat. *Am J Psych* 158: 1719.
- Gothelf D, Falk B, Singer P, Kairi M, Phillip M, et al. (2002) Weight Gain Associated With Increased Food Intake and Low Habitual Activity Levels in Male Adolescent Schizophrenic Inpatients Treated With Olanzapine. *Am J Psychiatry* 159: 1055–1057.
- Ryan MCM, Flanagan S, Kinsella U, Keeling F, Thakore JH (2004) The effects of atypical antipsychotics on visceral fat distribution in first episode, drug-naïve patients with schizophrenia. *Life Sci* 74: 1999–2008.
- Zhang Z-J, Yao Z-J, Liu WEN, Fang QUN, Reynolds GP (2004) Effects of antipsychotics on fat deposition and changes in leptin and insulin levels: Magnetic resonance imaging study of previously untreated people with schizophrenia. *Br J Psychiatry* 184: 58–62.
- Allison DB, Newcomer JW, Dunn AL, Blumenthal JA, Fabricatore AN, et al. (2009) Obesity Among Those with Mental Disorders: A National Institute of Mental Health Meeting Report. *Am J Prev Med* 36: 341–350.
- Brown S, Birtwistle J, Roe L, Thompson G (1999) The unhealthy lifestyle of people with schizophrenia. *Psychol Med* 29: 697–701.
- Archie S, Wilson J, Osborne S, Hobbs H, McNiven J (2003) Pilot study: access to fitness facility and exercise levels in olanzapine-treated patients. *Can J Psychiatry* 48: 628–632.
- Rader DJ (2007) Effect of Insulin Resistance, Dyslipidemia, and Intra-abdominal Adiposity on the Development of Cardiovascular Disease and Diabetes Mellitus. *Am J Med* 120: S12–S18.
- Weiden PJ, Mackell JA, McDonnell DD (2004) Obesity as a risk factor for antipsychotic noncompliance. *Schizophr Res* 66: 51–57.

19. Coccarello R, Moles A (2010) Potential mechanisms of atypical antipsychotic-induced metabolic derangement: Clues for understanding obesity and novel drug design. *Pharmacol Ther* 127: 210–251.
20. Sturtenberg FCJ, Bogers JPAM (2009) How can antipsychotics cause diabetes mellitus? Insights based on receptor-binding profiles, humoral factors and transporter proteins. *Eur Psychiat* 24: 164–170.
21. Reynolds GP, Kirk SL (2010) Metabolic side effects of antipsychotic drug treatment - pharmacological mechanisms. *Pharmacol Ther* 125: 169–179.
22. Deng C, Weston-Green K, Huang X-F (2010) The role of histaminergic H1 and H3 receptors in food intake: A mechanism for atypical antipsychotic-induced weight gain? *Prog Neuropsychopharmacol Biol Psychiatry* 34: 1–4.
23. Kim SF, Huang AS, Snowman AM, Teuscher C, Snyder SH (2007) Antipsychotic drug-induced weight gain mediated by histamine H1 receptor-linked activation of hypothalamic AMP-kinase. *Proceedings of the National Academy of Sciences* 104: 3456–3459.
24. Kirk S, Glazebrook J, Grayson B, Neill J, Reynolds G (2009) Olanzapine-induced weight gain in the rat role of 5-HT2C and histamine H1 receptors. *Psychopharmacology* 207: 119–125.
25. Matsui-Sakata A, Ohtani H, Sawada Y (2005) Receptor occupancy-based analysis of the contributions of various receptors to antipsychotics-induced weight gain and diabetes mellitus. *Drug Metabolism and Pharmacokinetics* 20: 368–378.
26. Nasrallah H (2008) Atypical antipsychotic-induced metabolic side effects: insights from receptor-binding profiles. *Mol Psychiat* 13: 27–35.
27. Berthoud H-R (2002) Multiple neural systems controlling food intake and body weight. *Neurosci Biobehav Rev* 26: 393–428.
28. Broberger C, Hökfelt T (2001) Hypothalamic and vagal neuropeptide circuitries regulating food intake. *Physiol Behav* 74: 669–682.
29. Faulconbridge LF, Grill HJ, Kaplan JM, Daniels D (2008) Caudal brainstem delivery of ghrelin induces fos expression in the nucleus of the solitary tract, but not in the arcuate or paraventricular nuclei of the hypothalamus. *Brain Res* 1218: 151–157.
30. Derbenev AV, Stuart TC, Smith BN (2004) Cannabinoids suppress synaptic input to neurons of the rat dorsal motor nucleus of the vagus nerve. *J Physiol (Lond)* 559: 923–938.
31. Berrendero F, Romero J, García-Gil L, Suarez I, De la Cruz P, et al. (1996) Changes in cannabinoid receptor binding and mRNA levels in several brain regions of aged rats. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1407: 205–214.
32. Bermudez-Silva FJ, Viveros MP, McPartland JM, Rodriguez de Fonseca F (2010) The endocannabinoid system, eating behavior and energy homeostasis: The end or a new beginning? *Pharmacol Biochem Behav* 95: 375–382.
33. Tiwari AK, Zai CC, Likhodi O, Lisker A, Singh D, et al. (2010) A Common Polymorphism in the Cannabinoid Receptor 1 (CNR1) Gene is Associated with Antipsychotic-Induced Weight Gain in Schizophrenia. *Neuropsychopharmacology* 35: 1315–1324.
34. Secher A, Husum H, Holst B, Egerod KL, Møllerup E (2010) Risperidone Treatment Increases CB1 Receptor Binding in Rat Brain. *Neuroendocrinology* 91: 155.
35. Weston-Green K, Huang X-F, Han M, Deng C (2008) The effects of antipsychotics on the density of cannabinoid receptors in the dorsal vagal complex of rats: implications for olanzapine-induced weight gain. *Int J Neuropsychopharmacol* 11: 827–835.
36. Pertwee RG (1997) Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacol Ther* 74: 129–180.
37. Van Sickle MD, Duncan M, Kingsley PJ, Mouhate A, Urbani P, et al. (2005) Identification and Functional Characterization of Brainstem Cannabinoid CB2 Receptors. *Science* 310: 329–332.
38. Meeker RB, Myers RD (1980) GABA and glutamate: Possible metabolic intermediaries involved in the hypothalamic regulation of food intake. *Brain Res Bull* 5: 253–259.
39. Vong L, Ye C, Yang Z, Choi B, Chua S, et al. (2011) Leptin Action on GABAergic Neurons Prevents Obesity and Reduces Inhibitory Tone to POMC Neurons. *Neuron* 71: 142–154.
40. Meister B (2007) Neurotransmitters in key neurons of the hypothalamus that regulate feeding behavior and body weight. *Physiol Behav* 92: 263–271.
41. Akbarian S, Huang H-S (2006) Molecular and cellular mechanisms of altered GAD1/GAD67 expression in schizophrenia and related disorders. *Brain Research Reviews* 52: 293–304.
42. Kaufman DL, Houser CR, Tobin AJ (1991) Two Forms of the γ -Aminobutyric Acid Synthetic Enzyme Glutamate Decarboxylase Have Distinct Intraneuronal Distributions and Cofactor Interactions. *J Neurochem* 56: 720–723.
43. Esclapez M, Tillakaratne NJ, Kaufman DL, Tobin AJ, Houser CR (1994) Comparative localization of two forms of glutamic acid decarboxylase and their mRNAs in rat brain supports the concept of functional differences between the forms. *J Neurosci* 14: 1834–1855.
44. Martin DL, Rimvall K (1993) Regulation of γ -Aminobutyric Acid Synthesis in the Brain. *J Neurochem* 60: 395–407.
45. Feldblum S, Erlander MG, Tobin AJ (1993) Different distributions of GAD65 and GAD67 mRNAs suggest that the two glutamate decarboxylases play distinctive functional roles. *J Neurosci Res* 34: 689–706.
46. Schwartz MW, Woods SC, Porte D, Scelley RJ, Baskin DG (2000) Central nervous system control of food intake. *Nature* 404: 661–671.
47. Bronstein DM, Schafer MKH, Watson SJ, Akil H (1992) Evidence that [beta]-endorphin is synthesized in cells in the nucleus tractus solitarius: detection of POMC mRNA. *Brain Res* 587: 269–275.
48. Wai S, Kindler P, Lam E, Zhang A, Yew D (2004) Distribution of neuropeptide Y-immunoreactive neurons in the human brainstem, cerebellum, and cortex during development. *Cell Mol Neurobiol* 24: 667–684.
49. Wu Q, Palmiter RD (2011) GABAergic signaling by AgRP neurons prevents anorexia via a melanocortin-independent mechanism. *Eur J Pharmacol* 660: 21–27.
50. Beck B (2006) Neuropeptide Y in normal eating and in genetic and dietary-induced obesity. *Philosophical Transactions of the Royal Society B: Biological Sciences* 361: 1159–1185.
51. Heilig M, Murison R (1987) Intracerebroventricular neuropeptide Y suppresses open field and home cage activity in the rat. *Regul Pept* 19: 221–231.
52. Heilig M, Wahlestedt C, Widerlöv E (1988) Neuropeptide Y (NPY)-induced suppression of activity in the rat: evidence for NPY receptor heterogeneity and for interaction with [alpha]-adrenoceptors. *Eur J Pharmacol* 157: 205–213.
53. Beck B, Strickerkrongrad A, Nicolas JP, Burlet C (1992) Chronic and continuous intracerebroventricular infusion of Neuropeptide-Y in Long-Evans rats mimics the feeding-behavior of obese Zucker rats. *Int J Obes* 16: 295–302.
54. Stanley BG, Kyrkouli SE, Lampert S, Leibowitz SF (1986) Neuropeptide Y chronically injected into the hypothalamus: A powerful neurochemical inducer of hyperphagia and obesity. *Peptides* 7: 1189–1192.
55. Obuchowicz E (1996) Long-term treatment with chlorpromazine and haloperidol but not with sulpiride and clozapine markedly elevates neuropeptide Y-like immunoreactivity in the rat hypothalamus. *Neuropeptides* 30: 471–478.
56. Kirk SL, Cahir M, Reynolds GP (2006) Clozapine, but not haloperidol, increases neuropeptide Y neuronal expression in the rat hypothalamus. *J Psychopharmacol (Oxf)* 20: 577–579.
57. Baptista T, Araujo de Baptista E, Ying Kin NMKN, Beaulieu S, Walker D, et al. (2002) Comparative effects of the antipsychotics sulpiride or risperidone in rats: I. Bodyweight, food intake, body composition, hormones and glucose tolerance. *Brain Res* 957: 144–151.
58. Pouzet B, Mow T, Kreilgärd M, Velschow S (2003) Chronic treatment with antipsychotics in rats as a model for antipsychotic-induced weight gain in human. *Pharmacol Biochem Behav* 75: 133–140.
59. Minet-Ringuet J, Even PC, Goubert M, Tomé D, de Beaupre R (2006) Long term treatment with olanzapine mixed with the food in male rats induces body fat deposition with no increase in body weight and no thermogenic alteration. *Appetite* 46: 254–262.
60. Minet-Ringuet J, Even PC, Guesdon B, Tomé D, de Beaupre R (2005) Effects of chronic neuroleptic treatments on nutrient selection, body weight, and body composition in the male rat under dietary self-selection. *Behav Brain Res* 163: 204–211.
61. Obuchowicz E, Krysiak R, Wieronska JM, Smalowska M, Herman ZS (2005) Alterations in striatal neuropeptide Y system activity of rats with haloperidol-induced behavioral supersensitivity. *Neuropeptides* 39: 515–523.
62. Obuchowicz E, Turchan J (1998) Influence of typical and atypical antipsychotics on neuropeptide Y-like immunoreactivity and NPY mRNA expression in rat striatum. *Neuropeptides* 32: 473–480.
63. Obuchowicz E, Turchan J (1999) Clozapine decreases neuropeptide Y-like immunoreactivity and neuropeptide Y mRNA levels in rat nucleus accumbens. *Eur Neuropsychopharmacol* 9: 329–335.
64. Obuchowicz E, Krysiak R, Herman ZS (2004) Does neuropeptide Y (NPY) mediate the effects of psychotropic drugs? *Neurosci Biobehav Rev* 28: 595–610.
65. Huang XF, Deng C, Zavitsanos K (2006) Neuropeptide Y mRNA expression levels following chronic olanzapine, clozapine and haloperidol administration in rats. *Neuropeptides* 40: 213–219.
66. Ferno J, Varela L, Skrede S, Vazquez MJ, Nogueiras R, et al. (2011) Olanzapine-Induced Hyperphagia and Weight Gain Associate with Orexigenic Hypothalamic Neuropeptide Signaling without Concomitant AMPK Phosphorylation. *PLoS ONE* 6: e20571.
67. Davoodi N, Kalinichev M, Korneev S, Clifton P (2009) Hyperphagia and increased meal size are responsible for weight gain in rats treated sub-chronically with olanzapine. *Psychopharmacology* 203: 693–702.
68. Ota M, Mori K, Nakashima A, Kaneko Y, Fujiwara K, et al. (2002) Peripheral injection of risperidone, an atypical antipsychotic, alters the bodyweight gain of rats. *Clin Exp Pharmacol Physiol* 29: 980–989.
69. Hartfield A, Moore N, Clifton P (2003) Effects of clozapine, olanzapine and haloperidol on the microstructure of ingestive behaviour in the rat. *Psychopharmacology* 167: 115.
70. Weston-Green K, Huang X-F, Deng C (2011) Olanzapine treatment and metabolic dysfunction: a dose response study in female Sprague Dawley rats. *Behav Brain Res* 217: 337–346.
71. Nemeroff C (1997) Dosing the antipsychotic medication olanzapine. *J Clin Psychiatry* 58: 45–49.
72. Albaugh V, Henry C, Bello N, Hajnal A, Lynch S, et al. (2006) Hormonal and metabolic effects of olanzapine and clozapine related to body weight in rodents. *Obesity* 14: 36–50.

73. Cope MB, Nagy TR, Fernandez JR, Geary N, Casey DE, et al. (2005) Antipsychotic drug-induced weight gain: development of an animal model. *Int J Obes Relat Metab Disord* 29: 607–614.
74. Kapur S, VanderSpek SC, Brownlee BA, Nobrega J (2003) Antipsychotic Dosing in Preclinical Models Is Often Unrepresentative of the Clinical Condition: A Suggested Solution Based on in Vivo Occupancy. *J Pharmacol Exp Ther* 305: 625–631.
75. Aravagiri M, Teper Y, Marder SR (1999) Pharmacokinetics and tissue distribution of olanzapine in rats. *Biopharm Drug Dispos* 20: 369–377.
76. Tauscher J, Jones C, Remington G, Zipursky R, Kapur S (2002) Significant dissociation of brain and plasma kinetics with antipsychotics. *Mol Psychiatr* 7: 317–321.
77. Kapur S, Wadenberg ML, Remington G (2000) Are animal studies of antipsychotics appropriately dosed? Lessons from the bedside to the bench. *Canadian Journal Of Psychiatry Revue Canadienne De Psychiatrie* 45: 241–246.
78. Deng C, Weston-Green KL, Han M, Huang X-F (2007) Olanzapine treatment decreases the density of muscarinic M2 receptors in the dorsal vagal complex of rats. *Prog Neuropsychopharmacol Biol Psychiatry* 31: 915–920.
79. Han M, Deng C, Burne THJ, Newell KA, Huang X-F (2008) Short- and long-term effects of antipsychotic drug treatment on weight gain and H1 receptor expression. *Psychoneuroendocrinology* 33: 569–580.
80. Huang X-F, Han M, Huang X, Zavitsanos K, Deng C (2006) Olanzapine differentially affects 5-HT_{2A} and 2C receptor mRNA expression in the rat brain. *Behav Brain Res* 171: 355–362.
81. Reagan-Shaw S, Nihal M, Ahmad N (2007) Dose translation from animal to human studies revisited. *The FASEB Journal* 22: 659–661.
82. Lin S, Storlien LH, Huang X-F (2000) Leptin receptor, NPY, POMC mRNA expression in the diet-induced obese mouse brain. *Brain Res* 875: 89–95.
83. Ling LL, Hughes LF, Caspary DM (2005) Age-related loss of the GABA synthetic enzyme glutamic acid decarboxylase in rat primary auditory cortex. *Neuroscience* 132: 1103–1113.
84. Deng C, Han M, Huang X (2007) No changes in densities of cannabinoid receptors in the superior temporal gyrus in schizophrenia. *Neurosci Bull* 23: 341–347.
85. Paxinos G, Watson C (2007) *The Rat Brain in Stereotaxic Coordinates*. CA, USA: Elsevier Academic Press Inc.
86. Kim E-M, O'Hare E, Grace MK, Welch CC, Billington CJ, et al. (2000) ARC POMC mRNA and PVN [alpha]-MSH are lower in obese relative to lean Zucker rats. *Brain Res* 862: 11–16.
87. Guan X-M, Yu H, Van der Ploeg LHT (1998) Evidence of altered hypothalamic pro-opiomelanocortin/neuropeptide Y mRNA expression in tubby mice. *Mol Brain Res* 59: 273–279.
88. Huang X-F, Han M, South T, Storlien L (2003) Altered levels of POMC, AgRP and MCH-R mRNA expression in the hypothalamus and other parts of the limbic system of mice prone or resistant to chronic high-energy diet-induced obesity. *Brain Res* 992: 9–19.
89. Krude H, Biebermann H, Luck W, Horn R, Brabant G, et al. (1998) Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. *Nat Genet* 19: 155–157.
90. Martin NM, Small CJ, Sajedi A, Liao XH, Weiss RE, et al. (2004) Abnormalities of the hypothalamo-pituitary-thyroid axis in the pro-opiomelanocortin deficient mouse. *Regul Pept* 122: 169–172.
91. Cone R (2005) Anatomy and regulation of the central melanocortin system. *Nat Neurosci* 8: 571–578.
92. Meister B, Göndü B, Suarez E, Ishii Y, Dürr K, et al. (2006) Hypothalamic proopiomelanocortin (POMC) neurons have a cholinergic phenotype. *Eur J Neurosci* 24: 2731–2740.
93. Zhang Y, Rodrigues E, Gao YX, King M, Cheng KY, et al. (2010) Pro-opiomelanocortin gene transfer to the nucleus of the solitary tract but not arcuate nucleus ameliorates chronic diet-induced obesity. *Neuroscience* 169: 1662–1671.
94. Ste Marie L, Luquet S, Cole T, Palmiter R (2005) Modulation of neuropeptide Y expression in adult mice does not affect feeding. *Proc Natl Acad Sci U S A* 102: 18632–18637. Epub 2005 Dec 18638.
95. Erickson JC, Clegg KE, Palmiter RD (1996) Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature* 381: 415–418.
96. Hollopeter G, Erickson JC, Seeley RJ, Marsh DJ, Palmiter RD (1998) Response of neuropeptide Y-deficient mice to feeding effectors. *Regul Pept* 75: 383–389.
97. Lin S, Boey D, Herzog H (2004) NPY and Y receptors: lessons from transgenic and knockout models. *Neuropeptides* 38: 189–200.
98. Cowley MA, Smart JL, Rubinstein M, Cerdan MG, Diano S, et al. (2001) Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* 411: 480.
99. Cifra A, Göres TJ, Palkovits M (1990) Neuropeptide Y innervation of ACTH-immunoreactive neurons in the arcuate nucleus of rats: a correlated light and electron microscopic double immunolabeling study. *Brain Res* 506: 215–222.
100. Roseberry AG, Liu H, Jackson AC, Cai X, Friedman JM (2004) Neuropeptide Y-Mediated Inhibition of Proopiomelanocortin Neurons in the Arcuate Nucleus Shows Enhanced Desensitization in ob/ob Mice. *Neuron* 41: 711–722.
101. Qian S, Chen H, Weingarth D, Trumbauer ME, Novi DE, et al. (2002) Neither Agouti-Related Protein nor Neuropeptide Y Is Critically Required for the Regulation of Energy Homeostasis in Mice. *Molecular Cell Biology* 22: 5027–5035.
102. Gropp E, Shanabrough M, Borok E, Xu AW, Janoschek R, et al. (2005) Agouti-related peptide-expressing neurons are mandatory for feeding. *Nat Neurosci* 8: 1289–1291.
103. Tong Q, Ye C-P, Jones JE, Elmquist JK, Lowell BB (2008) Synaptic release of GABA by AgRP neurons is required for normal regulation of energy balance. *Nat Neurosci* 11: 998–1000.
104. Hengges ST, Nishiyama M, Overstreet LS, Stenzel-Poore M, Williams JT, et al. (2004) GABA Release from Proopiomelanocortin Neurons. *J Neurosci* 24: 1578–1583.
105. Horvath TL, Bechmann I, Naeflin F, Kalra SP, Lanthorn C (1997) Heterogeneity in the neuropeptide Y-containing neurons of the rat arcuate nucleus: GABAergic and non-GABAergic subpopulations. *Brain Res* 756: 283–286.
106. Howlett AC, Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, et al. (2004) Cannabinoid physiology and pharmacology: 30 years of progress. *Neuropharmacology* 47: 345–358.
107. Di Marzo V, Goparaju SK, Wang L, Liu J, Batkai S, et al. (2001) Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 410: 822–825.
108. Alger BE (2002) Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. *Progress in Neurobiology* 68: 247–286.
109. Wilson RI, Nicoll RA (2002) Endocannabinoid signaling in the brain. (Review: Neuroscience). *Science* 296: 678(675).
110. Hengges ST, Low MJ, Williams JT (2005) Differential Regulation of Synaptic Inputs by Constitutively Released Endocannabinoids and Exogenous Cannabinoids. *J Neurosci* 25: 9746–9751.
111. Nguyen Q, Wagner E (2007) Estrogen differentially modulates the cannabinoid-induced presynaptic inhibition of amino acid neurotransmission in proopiomelanocortin neurons of the arcuate nucleus. *Neuroendocrinology* 84: 123.
112. Menzies JRW, Ludwig M, Leng G (2010) Direct and Indirect Effects of Cannabinoids on in vitro GABA Release in the Rat Arcuate Nucleus. *J Neuroendocrinol* 22: 585–592.
113. Gamber KM, MacArthur H, Westfall TC (2005) Cannabinoids augment the release of neuropeptide Y in the rat hypothalamus. *Neuropharmacology* 49: 646–652.
114. Kroeze WK, Hufeisen SJ, Popadak BA, Renock SM, Steinberg S, et al. (2003) H1-Histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. *Neuropsychopharmacology* 28: 519–526.
115. Richelson E, Souder T (2000) Binding of antipsychotic drugs to human brain receptors: Focus on newer generation compounds. *Life Sci* 68: 29–39.
116. Yoshimatsu H (2006) The neuronal histamine H1 and pro-opiomelanocortin-melanocortin 4 receptors: Independent regulation of food intake and energy expenditure. *Peptides* 27: 326–332.
117. Kuo D-Y (2002) Co-administration of dopamine D1 and D2 agonists additively decreases daily food intake, body weight and hypothalamic neuropeptide Y level in rats. *J Biomed Sci* 9: 126–132.
118. Kuo D-Y (2006) Hypothalamic neuropeptide Y (NPY) and the attenuation of hyperphagia in streptozotocin diabetic rats treated with dopamine D1/D2 agonists. *British Journal of Pharmacology* 148: 640–647.
119. Pelletier G, Simard J (1991) Dopaminergic regulation of pre-proNPY mRNA levels in the rat arcuate nucleus. *Neurosci Lett* 127: 96–98.
120. Heister LK, Cowley MA, Tecott LH, Fan W, Low MJ, et al. (2002) Activation of Central Melanocortin Pathways by Fenduramine. *Science* 297: 609–611.
121. Qiu J, Xue C, Bosch MA, Murphy JG, Fan W, et al. (2007) Serotonin 5-Hydroxytryptamine_{2C} Receptor Signaling in Hypothalamic Proopiomelanocortin Neurons: Role in Energy Homeostasis in Females. *Mol Pharmacol* 72: 885–896.
122. Bymaster F, Calligaro D, Falcone J, Marsh R, Moore N, et al. (1996) Radioreceptor binding profile of the atypical antipsychotic olanzapine. *Neuropsychopharmacology* 14: 87–96.
123. Bymaster F, Hemrick-Luecke S, Perry K, Fuller R (1996) Neurochemical evidence for antagonism by olanzapine of dopamine, serotonin, alpha₁-adrenergic and muscarinic receptors in vivo in rats. *Psychopharmacology* 124: 87–94.
124. Xu B, Kalra PS, Farmerie WG, Kalra SP (1999) Daily Changes in Hypothalamic Gene Expression of Neuropeptide Y, Galanin, Proopiomelanocortin, and Adipocyte Leptin Gene Expression and Secretion: Effects of Food Restriction. *Endocrinology* 140: 2868–2875.
125. Nasrallah H (2003) A review of the effect of atypical antipsychotics on weight. *Psychoneuroendocrinology* 28: 83–96.
126. NIMH Psychoactive Drug Screening Program website. Available: <http://pdsp.med.unc.edu/kidb.php>. Accessed 2011 Oct 20.
127. Roth B, Kroeze W, Patel S, Lopez E (2000) The Multiplicity of Serotonin Receptors: Uselessly diverse molecules or an embarrassment of riches? *The Neuroscientist* 6: 252–262.
128. Li C, Jones PM, Persaud SJ (2011) Role of the endocannabinoid system in food intake, energy homeostasis and regulation of the endocrine pancreas. *Pharmacol Ther* 129: 307–320.

129. Cavauto P, Witter GA (2009) The role of the endocannabinoid system in the regulation of energy expenditure. *Best Pract Res Clin En* 23: 79–86.
130. Nogueiras R, Diaz-Arteaga A, Lockie SH, Velásquez DA, Tschöp J, et al. (2009) The endocannabinoid system: Role in glucose and energy metabolism. *Pharmacol Res* 60: 93–98.
131. Harrold J, Williams G (2003) The cannabinoid system: a role in both the homeostatic and hedonic control of eating? *Br J Nutr* 90: 729–734.
132. Kirkham TC (2009) Cannabinoids and appetite: Food craving and food pleasure. *Int Rev Psychiatr* 21: 163–171.
133. Gomez R, Navarro M, Ferrer B, Trigo JM, Bilbao A, et al. (2002) A Peripheral Mechanism for CB1 Cannabinoid Receptor-Dependent Modulation of Feeding. *J Neurosci* 22: 9612–9617.
134. Cota D, Marsicano G, Tschöp M, Glubler Y, Flachskamm C, et al. (2003) The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest* 112: 423–431.
135. Sharkey KA, Pittman QJ (2005) Central and Peripheral Signaling Mechanisms Involved in Endocannabinoid Regulation of Feeding: A Perspective on the Munchies. *Sci STKE*. p15 p.
136. Kirkham T (2008) Endocannabinoids and the neurochemistry of gluttony. *J Neuroendocrinol* 20: 1099–1100.
137. Balcombe J, Barnard N, Sandusky G (2004) Laboratory routines cause animal stress. *Contemp Top Lab Anim* 43: 42–51.
138. Remington G, Mann S, McCormick P, Nobrega JN, Hahn M, et al. (2011) Modeling chronic olanzapine exposure using osmotic minipumps: Pharmacological limitations. *Pharmacology Biochemistry and Behavior* 100: 86–89.
139. van der Zwaal EM, Luijendijk MCM, Adan RAH, la Fleur SE (2008) Olanzapine-induced weight gain: Chronic infusion using osmotic minipumps does not result in stable plasma levels due to degradation of olanzapine in solution. *Eur J Pharmacol* 585: 130–136.
140. de Meijer VE, Le HD, Meisel JA, Puder M (2010) Repetitive orogastric gavage affects the phenotype of diet-induced obese mice. *Physiol Behav* 100: 387–393.
141. Beasley JCM, Tollefson G, Tran P, Satterlee W, Sanger T, et al. (1996) Olanzapine versus placebo and haloperidol: Acute phase results of the North American double-blind olanzapine trial. *Neuropsychopharmacology* 14: 111–123.

CHAPTER FIVE

5.1 OVERALL DISCUSSION AND CONCLUSIONS

The present series of studies demonstrate that olanzapine-induced metabolic side-effects are associated with alterations to muscarinic, melanocortinergic, GABAergic and cannabinoid neurotransmitter systems in the hypothalamus and brainstem. In addition, these studies show that multiple aspects of clinically-reported metabolic dysfunction during olanzapine treatment can be modelled in the female rat and occur in a largely dose-sensitive manner. Overall, these studies indicate that disruption to neurotransmitter signalling pathways involved in regulating appetite, glucose homeostasis and body weight may underlie a shift in energy balance that favours weight gain and insulin dysregulation during olanzapine treatment. Changes in the parameters examined occurred within 14 days' treatment, when a sharp increase in body weight is observed in the rat (Han et al., 2008a; Huang et al., 2006a). These findings contribute novel data towards understanding the mechanisms underlying olanzapine-induced metabolic side-effects and provide direction on optimal dosage for future animal modelling studies. This chapter will provide a general discussion of the findings and the potential mechanisms for olanzapine-induced metabolic dysfunction based on interactions between the systems examined (illustrated in Figure 5.1, pages 99-100). A detailed discussion of each study has been included at the end of Chapters 2-4. The main findings of this thesis are summarised in Table 5.1.

5.1.1 The Mechanisms of Olanzapine-Induced Metabolic Dysfunction

In Chapter 2 it was found that olanzapine self-administered in a sweet cookie dough pellet at 8-hourly intervals (t.i.d.) for 14 days induced metabolic side-effects in female Sprague Dawley rats

Table 5.1: Summary of Main Findings in Chapters 2-4

Parameter	Olanzapine Dosage Effect			
	0.25mg/kg	0.5mg/kg	1.0mg/kg	2.0mg/kg
<i>Total Body Weight Gained (g)</i>	NSC	↑*	↑*	↑**
<i>Consumption</i>				
Food Intake (g)	NSC	NSC	NSC	↑**
Feeding Efficiency [#]	NSC	↑*	↑**	↑**
Water Intake (ml)	NSC	NSC	NSC	NSC
<i>Adiposity (Fat Mass, g)</i>				
Inguinal	NSC	NSC	↑**	↑**
Perirenal	NSC	NSC	NSC	↑*
Periovary	NSC	NSC	NSC	NSC
Total White Fat	NSC	NSC	↑**	↑**
Subscapular Brown Fat	NSC	NSC	NSC	NSC
<i>Plasma Hormone Levels (fmol/ml)</i>				
Insulin	↓**	↓**	↓**	↓**
Total Ghrelin	↑*	↑**	↑*	↑*
Cholecystokinin	NSC	↑**	NSC	↑**
Peptide YY ₍₃₋₃₆₎	NSC	NSC	NSC	NSC
<i>Behaviour – Open Field</i>				
Total Distance Travelled (cm)	NSC	↓*	↓**	↓**^
Mean Velocity (cm/s)	NSC	↓**	↓**	↓**
Rearing Frequency	NSC	↓*	↓**	↓**
Centre Duration (s)	NSC	NSC	↓**	↓**
Periphery Duration (s)	NSC	NSC	↑**	↑**
Centre Frequency	NSC	NSC	↓**	↓**
Periphery Frequency	NSC	NSC	↓**	↓**
<i>M3R Binding Density (fmol/mg tissue)</i>				
Arcuate Nucleus	↑ (t)	↑**	↑**	↑**
Ventromedial Hypothalamic Nuclei	↑*	↑**	↑**	↑**
Dorsal Vagal Complex	NSC	↑*	↑**	↑**
<i>POMC mRNA Expression (nCi/g tissue)</i>				
Arcuate Nucleus	NSC	↓**	↓**	↓**
Dorsal Vagal Complex	NSC	NSC	NSC	NSC
<i>GAD₆₅ mRNA Expression (nCi/g tissue)</i>				
Arcuate Nucleus	NSC	NSC	↑*	↑**
Dorsal Vagal Complex	NSC	NSC	↑**	↑**
<i>CB1R Binding Density (fmol/mg tissue)</i>				
Arcuate Nucleus	↓**	↓**	↓**	↓**
Dorsal Vagal Complex	NSC	↓*	↓*	↓*
<i>NPY mRNA Expression (nCi/g tissue)</i>				
Arcuate Nucleus	NSC	NSC	↑**	↑**
Ventromedial Hypothalamic Nuclei	NSC	NSC	NSC	NSC
Dorsal Vagal Complex	NSC	NSC	NSC	NSC

Abbreviations: CB1R: cannabinoid CB1 receptor, GAD₆₅: glutamic acid decarboxylase isoform 65, M3R: muscarinic M3 receptor, NSC: no significant change, NPY: neuropeptide Y, POMC: pro-opiomelanocortin. [#](g weight gained / g food intake), **p*<0.05 vs. control, ***p*<0.01 vs. control, *t*=trend, *p*=0.053.

including weight gain, hyperphagia, enhanced feeding efficiency, increased intra-abdominal and subcutaneous adiposity, and decreased gross motor activity in an open-field paradigm. Olanzapine also altered circulating plasma metabolic hormones, i.e. decreased insulin, increased ghrelin and CCK levels, but had no effect on PYY₍₃₋₃₆₎ levels. These findings demonstrate that metabolic side-effects induced by olanzapine can be modelled in the rat in a manner that is consistent with reports from the clinic (Alvarez-Jimenez et al., 2006; Brown et al., 1999; Chiu et al., 2006; Eder et al., 2001; Gothelf et al., 2002; Murashita et al., 2007; Nemeroff, 1997; Ryan et al., 2004; Vidarsdottir et al., 2010), and validate this animal model as a solid foundation to investigate the effects of olanzapine on key candidate neurotransmission systems in the brain, *in-vivo*, in order to understand the underlying mechanisms of olanzapine-induced metabolic dysfunction.

As discussed in Chapters 1 and 3, several lines of evidence point to a critical role for the muscarinic M3R in the production and secretion of insulin via vagal innervation of the pancreas (Duttaroy et al., 2004; Gautam et al., 2006b; Gautam et al., 2008; Gromada and Hughes, 2006; Jindal and Keshavan, 2006; Ruiz de Azua et al., 2011). However, olanzapine is a potent antagonist of the muscarinic M3R (Johnson et al., 2005). In Chapter 3, it was reported for the first time that olanzapine acts on M3Rs in the hypothalamic VMH and Arc, and the DVC of the caudal brainstem, resulting in a compensatory increase in M3R binding density in these regions (Figure 5.1). Given the important role of the M3R in facilitating insulin secretion, it is interesting that alterations in M3R binding density correlated with reduced plasma insulin levels. My findings support a novel mechanism for olanzapine-induced insulin dysregulation whereby direct blockade of the M3R by olanzapine in the brainstem and hypothalamus inhibits the vagally-mediated ACh pathway for insulin secretion. Insulin dysregulation due to M3R blockade by olanzapine during the sub-chronic phase of treatment may be the initial insult that leads to insulin-resistance and type II

diabetes mellitus reported in the clinic during chronic treatment (Lambert et al., 2006; Lykkegaard et al., 2008; Sernyak et al., 2002). Clinical studies have reported the onset of type II diabetes mellitus in olanzapine-treated individuals that did not experience concurrent gains in body weight (Lambert et al., 2006; Ramankutty, 2002). Importantly, my data shows that M3R blockade by olanzapine can be a mechanism for type II diabetes independent of body weight gain as insulin dysregulation and M3R density changes were observed in the lowest olanzapine dosage group (0.25mg/kg olanzapine), which did not exhibit significant body weight gain. In addition to insulin levels, M3R binding density also correlated with plasma ghrelin and CCK levels. Studies have shown that the vagus nerve exerts inhibitory tone over ghrelin secretion (Lee et al., 2002; Toshinai et al., 2001) and that ghrelin secretion can be regulated by ACh (Shrestha et al., 2009), although not through the M1 or M4 receptor sub-types as pirenzepine has no effect on ghrelin levels (Broglio et al., 2004). In addition, a vagotomy enhances CCK levels in humans (Liddle, 2000), however vagal-cholinergic inhibition attenuates CCK satiation (Smith et al., 1981). My findings suggest that cholinergic M3R blockade by olanzapine may contribute to an increase in ghrelin and CCK levels, but inhibits the satiating pathway of CCK, through impaired vagal cholinergic signalling pathways. Collectively, the results of Chapter 3 show for the first time that olanzapine's antagonism of the M3R is associated with altered levels of metabolic hormones that signal appetite and the body's nutritional status to the brain, such as ghrelin, insulin and CCK, possibly through impaired vagal cholinergic signalling pathways to the GI tract (Figure 5.1).

A further novel contribution towards understanding the mechanisms underlying olanzapine-induced weight gain was the identification of changes to POMC/NPY and CB1R/GABA microcircuitry in the hypothalamus in a manner that favours weight gain during olanzapine treatment (Chapter 4). Olanzapine reduced anorexigenic POMC and increased orexigenic NPY mRNA expression in the hypothalamus, while concurrently reducing CB1R binding density and

enhancing GAD₆₅ mRNA expression in both the hypothalamus and brainstem. An increase in hypothalamic NPY and reduction in POMC mRNA expression can have profound effects on body weight through both sides of the energy equation, i.e. increased appetite and reduced activity (Heilig and Murison, 1987; Huo et al., 2009). Based on my findings, a new mechanism for olanzapine-induced weight gain involving interactions between local hypothalamic signalling pathways was proposed, whereby increased NPY and enhanced inhibitory GABAergic output, possibly through reduced CB1R density, contribute to POMC inhibition (see Figure 4.6, Chapter 4). My results demonstrate that olanzapine acts on a number of neurotransmitter signalling pathways in regions of the brain that regulate energy homeostasis. But the key question is: how do changes in hormonal signals interact with neurotransmission pathways, in particular POMC/NPY, in the hypothalamus and brainstem during olanzapine treatment? A possible link between peripheral and neural effects during olanzapine treatment is interactions with the M3R (Figure 5.1), since olanzapine has a very low/no affinity for GABA receptors or CB1Rs (Roth and Driscoll, 2011).

As discussed in Chapter 1, metabolic hormones exert their effects on the brain to convey information pertaining to the nutritional and energy status of the body, and thus, hypothalamic neuropeptide expression can be modulated by peripheral metabolic condition (see Section 1.2.3.B. Brain-Gut Axis, page 16). Insulin, ghrelin and CCK mediate their appetite effects, in part, by regulating NPY and/or POMC. For example, anorexigenic insulin enhances POMC mRNA expression and inhibits NPY in the Arc through insulin receptors expressed by these neurons (Plum et al., 2006), whereas expression of the NPY precursor molecule, prepro-NPY, is increased in the Arc of insulin-deficient streptozotocin-diabetic rats (White et al., 1990). Ghrelin inhibits POMC by enhancing inhibitory GABA release from NPY terminals (Cowley et al., 2003) and data from the present studies show a positive correlation between ghrelin and GAD₆₅ mRNA

expression in both the Arc and DVC ($r=0.51$, $p<0.05$ and $r=0.57$, $p<0.01$, respectively). CCK administration selectively activates POMC neurons in the NTS via the vagus nerve (Fan et al., 2004), however olanzapine did not affect POMC expression in the DVC. During olanzapine treatment impaired cholinergic signalling, i.e. through M3R blockade, may inhibit the ability of CCK to activate POMC in the NTS and induce satiety. Taken together, a reduction in insulin and increase in ghrelin during olanzapine treatment through M3R blockade may contribute to body weight gain by enhancing NPY and GABAergic inhibitory tone and suppressing POMC (Figure 5.1). Impaired cholinergic signalling pathways during olanzapine treatment may reduce satiety signalling to the brainstem. Moreover, my data shows that the M3R can indirectly influence hypothalamic neuropeptides by altering plasma hormone signalling to the brain (Figure 5.1); indeed, NPY and POMC mRNA expression positively correlated with M3R density in the Arc ($r=0.58$, $p<0.01$ and $r=0.51$, $p<0.01$, respectively) in the present study. Figure 5.1 illustrates a possible mechanism for olanzapine-induced weight gain and insulin dysregulation through interactions between systems examined in the present series of studies.

In addition to an indirect role for the M3R in olanzapine-induced metabolic dysfunction via hormone regulation, several studies demonstrate that the M3R plays a direct role in cannabinoid and GABAergic neurotransmission. A study by Marini et al., (2009) reported that pre-activation of the M3R in human neuroblastoma SH-SY5Y cells increases intracellular calcium levels up to 8-fold compared to CB1R activation alone. The idea that M3Rs work with CB1Rs to modulate intracellular calcium levels has interesting implications for the vesicular release of GABA and subsequent regulation of POMC (Figure 5.1). In the hippocampus, M1/M3R activation increases endogenous cannabinoid production, which suppresses GABA release via CB1R activation (Fukudome et al., 2004). On the other hand, M3R antagonism by olanzapine may reduce

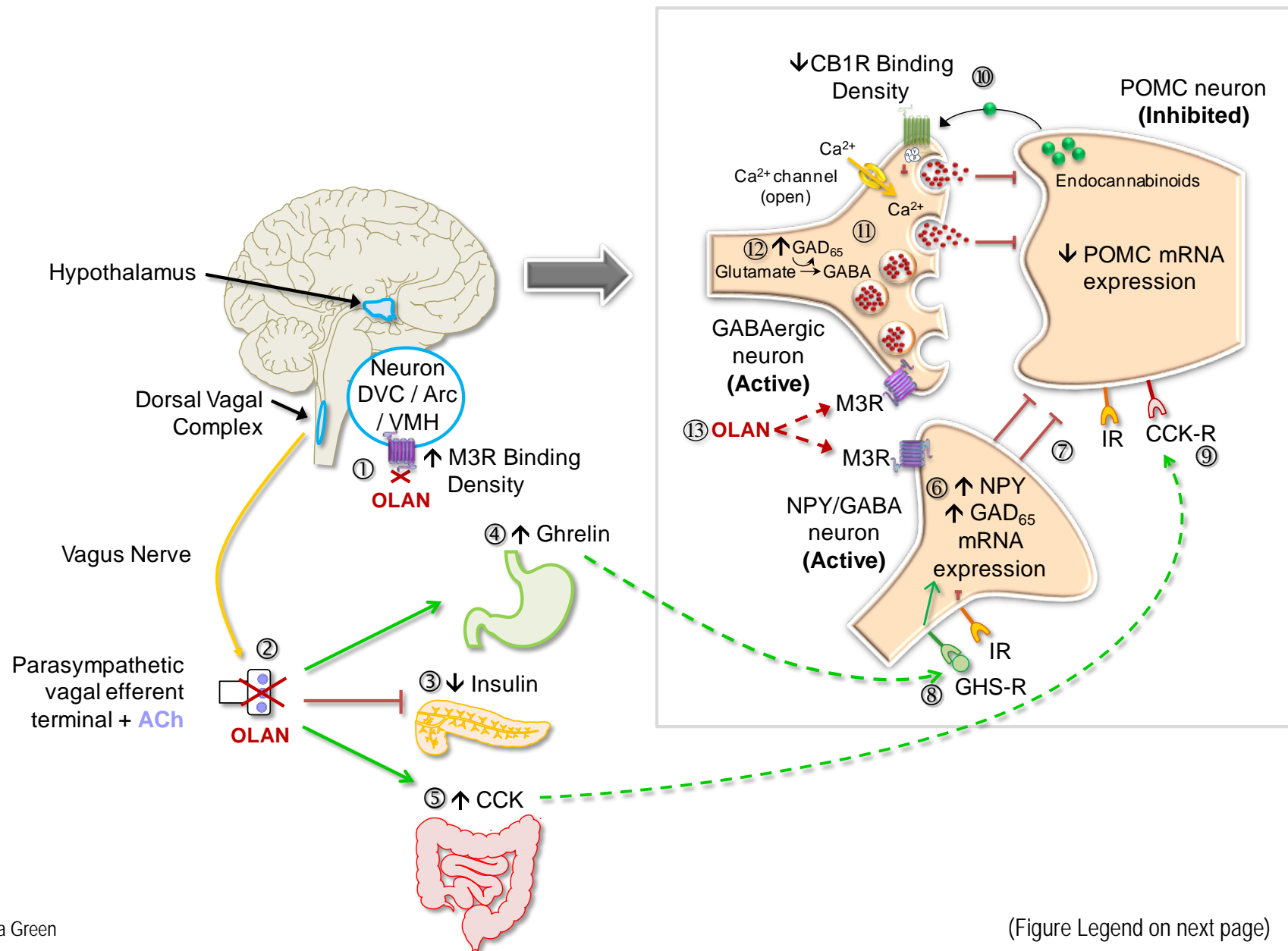


Figure 5.1: A Potential Mechanism Underlying Olanzapine-Induced Metabolic Dysfunction Based on this Study

M3R blockade by olanzapine causes a compensatory upregulation of M3R density in the Arc, VMH and DVC (1), and inhibits the ACh-mediated pathway of insulin secretion (2), causing hypoinsulinaemia (3). Interference in cholinergic vagal innervation of the stomach and intestines (2) causes an increase in orexigenic ghrelin (4) and anorexigenic CCK (5). Olanzapine increases NPY and GAD₆₅ mRNA expression (6), which inhibit anorexigenic POMC neurons (7). Activation of GHS-R on NPY neurons (8) contributes to GABAergic inhibition of POMC (7). Hypoinsulinaemia during olanzapine treatment reduces inhibition of NPY and activation of POMC. Vagal cholinergic inhibition during olanzapine treatment, via M3R blockade, may prevent CCK activation of POMC in the NTS (9). POMC neurons in the Arc express endogenous cannabinoids, which diffuse in a retrograde manner to activate CB1Rs expressed on GABAergic neurons (10). Activation of the CB1R stimulates the G-protein coupled $\beta\gamma$ subunit to inhibit opening of Ca²⁺ channels (11), which reduces the probability of vesicular release of GABA from the axon terminal, disinhibiting POMC. However, olanzapine decreases CB1R density, allowing Ca²⁺ channels to open and enhance release of GABA onto the post-synaptic POMC neuron. Increased GABA synthesis during olanzapine treatment would allow greater inhibitory input to POMC (12). Collectively, olanzapine-induced alterations to insulin, ghrelin and CCK signalling to the brain, through M3R blockade, contributes to altered local regulation of hypothalamic POMC and NPY by GABAergic and cannabinoid neurotransmission in a manner that suppresses anorexigenic signalling and favours weight gain during the early stage* of olanzapine treatment. In addition, the M3R can directly interact with GABAergic neurotransmission by modulating endocannabinoid levels and subsequent activation of the CB1R (13).

Abbreviations: ACh: acetylcholine, Arc: arcuate nucleus, CCK: cholecystokinin, CCK-R: cholecystokinin receptor, DVC: dorsal vagal complex, GABA: γ -Aminobutyric acid, GAD₆₅: glutamic acid decarboxylase isoform 65, GHS-R: growth hormone secretagogue (ghrelin) receptor, IR: insulin receptor, M3R: muscarinic M3 receptor, NPY: neuropeptide Y, NTS: nucleus of the solitary tract, POMC: pro-opiomelanocortin, VMH: ventromedial hypothalamic nucleus. Picture modified from Weston-Green et al., PLoS One (*Manuscript Accepted 11th February, 2012, In Press*).

*Note: Chronic treatment with olanzapine can cause hyperinsulinaemia (discussed in Chapters 1 and 3).

cannabinoid production and enhance GABA release; however under conditions of low endogenous ligand, a compensatory increase in CB1R density over time would be expected.

Although olanzapine potently blocks the M3R, the functional outcome of increased M3R density throughout olanzapine treatment requires further examination. Given the complexity of energy homeostasis and the broad receptor binding profile of olanzapine, the mechanisms discussed in this chapter may contribute partially to the metabolic side-effects of olanzapine and more research is required. As discussed in Chapter 4, olanzapine is a histamine H1 receptor antagonist (Richelson and Souder, 2000) and antipsychotic affinity for the H1 receptor can predict its weight gain liability (Kroeze et al., 2003); however, the underlying mechanisms for the effect of the H1 receptor on antipsychotic-induced body weight gain may be independent of melanocortineric neurotransmission (Yoshimatsu, 2006). In addition, interactions between the melanocortineric system, and receptor sub-types of the dopaminergic and serotonergic neurotransmission systems have been demonstrated (Heisler et al., 2002; Kuo, 2002; Kuo, 2006; Pelletier and Simard, 1991; Qiu et al., 2007) and may contribute to olanzapine-induced metabolic side-effects (Kirk et al., 2009). Also, it is important to note that statistical correlations do not provide direct evidence of a causal link and further studies are required to confirm the proposed mechanisms contained within this series of studies. Nevertheless, these results have given some insight into the mechanisms underlying olanzapine-induced metabolic dysfunction.

5.1.2. Recommendations for Further Research

Based on the findings of the present series of studies, recommendations for further research are as follows:

1. Olanzapine treatment altered the mRNA expression of POMC, NPY and GAD₆₅, however levels of mRNA expression may not necessarily reflect the amount of protein. Western blot techniques may be utilised to examine changes on a protein level as well as effects on downstream pathways in a future study.
2. Olanzapine-induced alterations in CB1Rs and M3Rs were observed using receptor binding autoradiographic methods. Further studies are required to identify whether these changes were a result of receptor number or binding affinity, and whether the receptor changes reflected downstream signalling alterations. Functional binding assays using [³⁵S]GTP-γ-S binding can determine the function of GPCRs, while western blot techniques can detect downstream signalling proteins. However, the development of an M3R-specific ligand is required to accurately examine the binding affinity of the M3R.
3. NPY mRNA expression increased in the present study following 14 days' olanzapine treatment, whereas NPY mRNA expression did not change after 5 weeks' olanzapine treatment (Huang et al., 2006b). Although the studies differ in experimental design (i.e. 1.2mg/kg olanzapine administered once daily for 5 weeks), these findings may indicate a time-dependent sensitivity of NPY mRNA expression to olanzapine treatment that resembles the 'peak and plateau' trend observed in body weight gain in humans and rats. Further studies examining the time-course of NPY mRNA expression during olanzapine treatment will assist in clarifying this theory.
4. My results revealed hypoinsulinaemia, low blood glucose levels and an increase in M3R density following 2 weeks' olanzapine treatment, whereas it is well-documented that chronic olanzapine treatment is associated with hyperinsulinaemia and hyperglycaemia (Oriot et al.,

2008; Perez-Iglesias et al., 2008). Interestingly, a clinical study found that olanzapine induced a time-dependent change in insulin secretion over 8 weeks' treatment (Chiu et al., 2010). Therefore, further studies examining the effects of olanzapine on neuronal and pancreatic M3Rs, as well as insulin and glucose levels at several stages of treatment (acute, sub-chronic, chronic phases) would be useful. Investigation into glucose tolerance testing and insulin sensitivity over time should also be conducted.

5. Future development of an M3R-specific agonist may have clinical implications whereby co-treatment with olanzapine may be able to prevent or attenuate the initial insulin dysfunction induced by M3R blockade. Co-treatment with an M3R agonist may also alleviate imbalances in other metabolic hormones, such as ghrelin and CCK. Furthermore, several studies have shown that aspects of gluco-metabolic dysfunction can be modelled in the rat using clozapine (Murashita et al., 2007; Tulipano et al., 2007). Clozapine is also a potent M3R antagonist (Johnson et al., 2005) and has the highest diabetogenic/obesogenic liability of all SGAs (Newcomer, 2005); therefore M3R agonist co-treatment should also be examined in a pre-clinical model to test clinical viability. Importantly, removal of M3R antagonistic properties in future SGAs may be beneficial to reduce metabolic side-effects.
6. Male rats exhibit enhanced sensitivity to olanzapine-induced metabolic side-effects when the drug is self-administered via their food source (Minet-Ringuet et al., 2006a; Shobo et al., 2010). Therefore, utilising the experimental design employed in Chapter 2, i.e. dosage considerations, treatment interval and administration method, may be beneficial in enhancing response in the male rat for the development of a better male rat model.

5.1.3. Conclusion

The results of the present thesis have demonstrated that alterations to a number of major metabolic pathways in the brain and periphery contribute to olanzapine's metabolic side-effects. An olanzapine dosage response was apparent in most parameters measured (summarised in Table 5.1, page 94). Olanzapine-induced body weight gain is associated with hyperphagia, adiposity, altered circulating metabolic hormone levels and decreased locomotor activity. Olanzapine alters specific neuropeptides and neurotransmitter receptor systems in regions of the brain that regulate energy homeostasis through food intake and locomotor activity. Olanzapine increased M3R binding density in the hypothalamus and brainstem, which was likely to be a homeostatic upregulation due to the drug's profile to potently block the M3R. Olanzapine's antagonism of the M3R may interfere in the cholinergic pathway for GI regulation, contributing in part to its weight gain side-effects through interference in cholinergic vagal innervation of the GI tract, resulting in abnormal ghrelin and CCK levels. However, olanzapine's effects on the M3R may be more important to its diabetogenic liability through insulin dysregulation and resultant glucose homeostatic imbalance that precedes insulin-resistance and type II diabetes mellitus. The present study demonstrates a mechanism for olanzapine-induced insulin dysregulation that is independent of body weight gain. Olanzapine increases NPY and decreases POMC mRNA expression in the Arc, which may contribute to body weight gain by increasing appetite and reducing locomotor activity. In addition, olanzapine decreases CB1R binding density and increases the GABA synthesising enzyme, GAD₆₅, in the Arc and DVC. These alterations may interfere in the CB1R-mediated GABAergic inhibition of POMC neurons in the Arc, but through a different post-synaptic neuron in the DVC. Taken together, numerous aspects of metabolic dysfunction reported in the clinic during olanzapine treatment can be modelled in the female rat using clinically comparable drug dosages. This model provides a foundation for the examination of drug-effects *in vivo*, in order to understand the mechanisms by which olanzapine induces

obesity and type II diabetes mellitus side-effects. The present study offers insight into the mechanisms underlying antipsychotic-induced metabolic side-effects and novel directions for the design of new generation antipsychotic drugs that have reduced metabolic side-effects in order to enhance patient outcomes and quality of life.

REFERENCES

- (28 January 2000) *Science Netwatch*, 287: 5453. <http://pdsp.med.unc.edu/kidb.php>.
- Abbott, CR, Small, CJ, Kennedy, AR, Neary, NM, Sajedi, A, Ghatei, MA, Bloom, SR (2005) Blockade of the neuropeptide Y Y2 receptor with the specific antagonist BIIIE0246 attenuates the effect of endogenous and exogenous peptide YY(3-36) on food intake. *Brain Research*, 1043(1-2): 139-144.
- Ahren, B (2009) Islet G protein-coupled receptors as potential targets for treatment of type 2 diabetes. *Nature Reviews. Drug Discovery*, 8(5): 369-385.
- Ahrén, B (2000) Autonomic regulation of islet hormone secretion – Implications for health and disease. *Diabetologia*, 43(4): 393-410.
- Akbarian, S, Huang, H-S (2006) Molecular and cellular mechanisms of altered GAD1/GAD67 expression in schizophrenia and related disorders. *Brain Research Reviews*, 52(2): 293-304.
- Albaugh, V, Henry, C, Bello, N, Hajnal, A, Lynch, S, Halle, B, Lynch, C (2006) Hormonal and metabolic effects of olanzapine and clozapine related to body weight in rodents. *Obesity*, 14(1): 36-50.
- Albaugh, VL, Judson, JG, She, P, Lang, CH, Maresca, KP, Joyal, JL, Lynch, CJ (2010) Olanzapine promotes fat accumulation in male rats by decreasing physical activity, repartitioning energy and increasing adipose tissue lipogenesis while impairing lipolysis. *Molecular Psychiatry*, 16(5): 569-581.
- Alger, BE (2002) Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. *Progress in Neurobiology*, 68(4): 247-286.
- Allison, D, Mentore, J, Heo, M, Chandler, L, Cappelleri, J, Infante, M, PJ, W (1999) Antipsychotic-induced weight gain: a comprehensive research synthesis. *The American Journal of Psychiatry*, 156(11): 1686-1696.
- Allison, DB, Newcomer, JW, Dunn, AL, Blumenthal, JA, Fabricatore, AN, Daumit, GL, Cope, MB, Riley, WT, Vreeland, B, Hibbeln, JR, Alpert, JE (2009) Obesity Among Those with Mental Disorders: A National Institute of Mental Health Meeting Report. *American Journal of Preventive Medicine*, 36(4): 341-350.
- Alvarez-Jimenez, M, Gonzalez-Blanch, C, Vazquez-Barquero, JL, Perez-Iglesias, R, Martinez-Garcia, O, Perez-Pardal, T, Ramirez-Bonilla, ML, Crespo-Facorro, B (2006) Attenuation of antipsychotic-induced weight gain with early behavioral intervention in drug-naïve first-episode psychosis patients: A randomized controlled trial. *Journal of Clinical Psychiatry*, 67(8): 1253-1260.
- American Diabetes Association, American Psychiatric Association, American Association of Clinical Endocrinologists, Obesity, NAAftSo (2004) Consensus Development Conference on Antipsychotic Drugs and Obesity and Diabetes. *Diabetes Care*, 27(2): 596-601.
- Anderman, B, Griffith, RW (1977) Clozapine-induced agranulocytosis: A situation report up to August 1976. *European Journal of Clinical Pharmacology*, 11(3): 199-201.
- Andreasen, NC, Arndt, S, Alliger, R, Miller, D, Flaum, M (1995) Symptoms of Schizophrenia: Methods, Meanings, and Mechanisms. *Archives of General Psychiatry*, 52(5): 341-351.
- Antony, S, Kumar T, P, Mathew, J, Anju, TR, Paulose, CS (2010) Hypoglycemia induced changes in cholinergic receptor expression in the cerebellum of diabetic rats. *Journal of Biomedical Science*, 17(1): 7.

- Aravagiri, M, Teper, Y, Marder, SR (1999) Pharmacokinetics and tissue distribution of olanzapine in rats. *Biopharmaceutics and Drug Disposition*, 20(8): 369-377.
- Archie, S, Wilson, J, Osborne, S, Hobbs, H, McNiven, J (2003) Pilot study: access to fitness facility and exercise levels in olanzapine-treated patients. *Canadian Journal of Psychiatry*, 48(9): 628-632.
- Arjona, AA, Zhang, SX, Adamson, B, Wurtman, RJ (2004) An animal model of antipsychotic-induced weight gain. *Behavioural Brain Research*, 152(1): 121-127.
- Armstrong, DM, Manley, L, Haycock, JW, Hersh, LB (1990) Co-localization of choline acetyltransferase and tyrosine hydroxylase within neurons of the dorsal motor nucleus of the vagus. *Journal of Chemical Neuroanatomy*, 3(2): 133-140.
- Arner, P, Pollare, T, Lithell, H (1991) Different aetiologies of Type 2 (non-insulin-dependent) diabetes mellitus in obese and non-obese subjects. *Diabetologia*, 34(7): 483-487.
- Atmaca, M, Tezcan, E, Ustundag, B (2007) Plasma nitric oxide and leptin values in patients with olanzapine-induced weight gain. *Journal of Psychiatric Research*, 41(1-2): 74-79.
- Bachus, SE, Hyde, TM, Herman, MM, Egan, MF, Kleinman, JE (1997) Abnormal cholecystokinin mRNA levels in entorhinal cortex of schizophrenics. *Journal of Psychiatric Research*, 31(2): 233-256.
- Balakrishnan, S, Mathew, J, Antony, S, Paulose, CS (2009) Muscarinic M1, M3 receptors function in the brainstem of streptozotocin induced diabetic rats: Their role in insulin secretion from the pancreatic islets as a function of age. *European Journal of Pharmacology*, 608(1-3): 14-22.
- Balcombe, J, Barnard, N, Sandusky, C (2004) Laboratory routines cause animal stress. *Contemporary Topics in Laboratory Animal Science*, 43(6): 42-51.
- Ballard, C, Waite, J, Birks, J (2006) Atypical antipsychotics for aggression and psychosis in Alzheimer's disease. *Cochrane Database of Systematic Reviews 2006*, (Issue 1): Art. No.: CD003476. DOI: 003410.001002/14651858.CD14003476.pub14651852.
- Baptista, T, Araujo de Baptista, E, Ying Kin, NMKN, Beaulieu, S, Walker, D, Joobar, R, Lalonde, J, Richard, D (2002) Comparative effects of the antipsychotics sulpiride or risperidone in rats: I: Bodyweight, food intake, body composition, hormones and glucose tolerance. *Brain Research*, 957(1): 144-151.
- Baptista, T, Rangel, N, Fernández, V, Carrizo, E, El Fakih, Y, Uzcátegui, E, Galeazzi, T, Gutiérrez, MA, Servigna, M, Dávila, A, Uzcátegui, M, Serrano, A, Connell, L, Beaulieu, S, de Baptista, EA (2007) Metformin as an adjunctive treatment to control body weight and metabolic dysfunction during olanzapine administration: A multicentric, double-blind, placebo-controlled trial. *Schizophrenia Research*, 93(1-3): 99-108.
- Baskin, DG, Breininger, JF, Schwartz, MW (1999) Leptin receptor mRNA identifies a subpopulation of neuropeptide Y neurons activated by fasting in rat hypothalamus. *Diabetes*, 48(4): 828-833.
- Batterham, RL, Bloom, SR (2003) The gut hormone peptide YY regulates appetite. *Annals of the New York Academy of Sciences*, 994: 162-168.
- Batterham, RL, Cohen, MA, Ellis, SM, Le Roux, CW, Withers, DJ, Frost, GS, Ghatei, MA, Bloom, SR (2003) Inhibition of Food Intake in Obese Subjects by Peptide YY(3-36). *New England Journal of Medicine*, 349(10): 941-948.
- Batterham, RL, Cowley, MA, Small, CJ, Herzog, H, Cohen, MA, Dakin, CL, Wren, AM, Brynes, AE, Low, MJ, Ghatei, MA, Cone, RD, Bloom, SR (2002) Gut hormone PYY3-36 physiologically inhibits food intake. *Nature*, 418(6898): 650-654.
- Beasley, JCM, Tollefson, G, Tran, P, Satterlee, W, Sanger, T, Hamilton, S (1996) Olanzapine versus placebo and haloperidol: Acute phase results of the North American double-blind olanzapine trial. *Neuropsychopharmacology*, 14(2): 111-123.

- Beck, B (2006) Neuropeptide Y in normal eating and in genetic and dietary-induced obesity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 361(1471): 1159-1185.
- Beck, B, Burlet, A, Bazin, R, Nicolas, J, Burlet, C (1993) Elevated neuropeptide Y in the arcuate nucleus of young obese Zucker rats may contribute to the development of their overeating. *The Journal of Nutrition*, 123(6): 1168-1172.
- Beck, B, Strickerkrongrad, A, Nicolas, JP, Burlet, C (1992) Chronic and continuous intracerebroventricular infusion of Neuropeptide-Y in Long-Evans rats mimics the feeding-behavior of obese Zucker rats. *International Journal of Obesity*, 16(4): 295-302.
- Beebe, KL, Block, T, DeBattista, C, Blasey, C, Belanoff, JK (2006) The efficacy of mifepristone in the reduction and prevention of olanzapine-induced weight gain in rats. *Behavioural Brain Research*, 171(2): 225-229.
- Beinfeld, MC, Garver, DL (1991) Concentration of cholecystokinin in cerebrospinal fluid is decreased in psychosis: Relationship to symptoms and drug response. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 15(5): 601-609.
- Belfiore, F, Iannello, S (1998) Insulin Resistance in Obesity: Metabolic Mechanisms and Measurement Methods. *Molecular Genetics and Metabolism*, 65(2): 121-128.
- Bereiter, DA, Rohner-Jeanrenaud, F, Berthoud, H-R, Jeanrenaud, B (1981) CNS modulation of pancreatic endocrine function. *Diabetologia*, 20: 417-425.
- Bermudez-Silva, FJ, Viveros, MP, McPartland, JM, Rodriguez de Fonseca, F (2010) The endocannabinoid system, eating behavior and energy homeostasis: The end or a new beginning? *Pharmacology, Biochemistry and Behavior*, 95(4): 375-382.
- Berrendero, F, Romero, J, García-Gil, L, Suarez, I, De la Cruz, P, Ramos, JA, Fernández-Ruiz, JJ (1998) Changes in cannabinoid receptor binding and mRNA levels in several brain regions of aged rats. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1407(3): 205-214.
- Berridge, KC, Pecina, S (1995) Benzodiazepines, appetite, and taste palatability. *Neuroscience and Biobehavioral Reviews*, 19(1): 121-131.
- Berthoud, H-R (2002) Multiple neural systems controlling food intake and body weight. *Neuroscience and Biobehavioral Reviews*, 26(4): 393-428.
- Berthoud, H-R (2008) The vagus nerve, food intake and obesity. *Regulatory Peptides*, 149(1-3): 15-25.
- Berthoud, H-R, Jeanrenaud, B (1979) Acute hyperinsulinemia and its reversal by vagotomy after lesions of the ventromedial hypothalamus in anesthetized rats. *Endocrinology*, 105: 146-151.
- Berthoud, HR, Carlson, NR, Powley, TL (1991) Topography of efferent vagal innervation of the rat gastrointestinal tract. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 260(1): R200-R207.
- Bobes, J, Rejas, J, Garcia-Garcia, M, Rico-Villademoros, F, García-Portilla, MP, Fernández, I, Hernández, G (2003) Weight gain in patients with schizophrenia treated with risperidone, olanzapine, quetiapine or haloperidol: results of the EIRE study. *Schizophrenia Research*, 62(1-2): 77-88.
- Bouche, C, Serdy, S, Kahn, CR, Goldfine, AB (2004) The Cellular Fate of Glucose and Its Relevance in Type 2 Diabetes. *Endocrine Reviews*, 25(5): 807-830.
- Boyda, HN, Tse, L, Procyshyn, RM, Honer, WG, Barr, AM (2010a) Preclinical models of antipsychotic drug-induced metabolic side effects. *Trends in Pharmacological Sciences*, 31(10): 484-497.
- Boyda, HN, Tse, L, Procyshyn, RM, Wong, D, Wu, TKY, Pang, CC, Barr, AM (2010b) A parametric study of the acute effects of antipsychotic drugs on glucose sensitivity in an

- animal model. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 34(6): 945-954.
- Brambilla, F, Monteleone, P, Maj, M (2007) Olanzapine-induced weight gain in anorexia nervosa: Involvement of leptin and ghrelin secretion? *Psychoneuroendocrinology*, 32(4): 402-406.
- Bray, GA, Fisler, J, York, DA (1990) Neuroendocrine control of the development of obesity: understanding gained from studies of experimental animal models. *Frontiers in Neuroendocrinology*, 11: 128-181.
- Breier, A, Buchanan, R, Kirkpatrick, B, Davis, O, Irish, D, Summerfelt, A, Carpenter Jr., W (1994) Effects of clozapine on positive and negative symptoms in outpatients with schizophrenia. *The American Journal of Psychiatry*, 151(1): 20-26.
- Bridle, C, Palmer, S, Bagnall, AM, Darba, J, Duffy, S, Sculpher, M, Riemsma, R (2004) A rapid and systematic review and economic evaluation of the clinical and cost-effectiveness of newer drugs for treatment of mania associated with bipolar affective disorder. *Health Technology Assessment*, 8(19): iii-iv.
- Broberger, C, Hökfelt, T (2001) Hypothalamic and vagal neuropeptide circuitries regulating food intake. *Physiology and Behavior*, 74(4-5): 669-682.
- Broglia, F, Gottero, C, Van Koetsveld, P, Prodám, F, Destefanis, S, Benso, A, Gauna, C, Hofland, L, Arvat, E, van der Lely, AJ, Ghigo, E (2004) Acetylcholine Regulates Ghrelin Secretion in Humans. *Journal of Clinical Endocrinology and Metabolism*, 89(5): 2429-2433.
- Bronstein, DM, Schafer, MKH, Watson, SJ, Akil, H (1992) Evidence that [beta]-endorphin is synthesized in cells in the nucleus tractus solitarius: detection of POMC mRNA. *Brain Research*, 587(2): 269-275.
- Brown, S, Birtwistle, J, Roe, L, Thompson, C (1999) The unhealthy lifestyle of people with schizophrenia. *Psychological Medicine*, 29(3): 697-701.
- Buchan, AM, Polak, JM, Solcia, E, Capella, C, Hudson, D, Pearse, AG (1978) Electron immunohistochemical evidence for the human intestinal I cell as the source of CCK. *Gut*, 19(5): 403-407.
- Budman, C, Gayer, A, Lesser, M, Shi, Q, Bruun, R (2001) An open-label study of the treatment efficacy of olanzapine for Tourette's disorder. *Journal of Clinical Psychiatry*, 62(4): 290-294.
- Buijs, R, Chun, S, Nijima, A, Romijn, H, Nagai, K (2001) Parasympathetic and sympathetic control of the pancreas: A role for the suprachiasmatic nucleus and other hypothalamic centers that are involved in the regulation of food intake. *The Journal of Comparative Neurology*, 431(4): 405-423.
- Burcelin, R (2010) The gut-brain axis: a major glucoregulatory player. *Diabetes and Metabolism*, 36(Suppl. 3): S54-S58.
- Burdyga, G, Lal, S, Varro, A, Dimaline, R, Thompson, DG, Dockray, GJ (2004) Expression of Cannabinoid CB1 Receptors by Vagal Afferent Neurons Is Inhibited by Cholecystokinin. *Journal of Neuroscience*, 24(11): 2708-2715.
- Bymaster, F, Calligaro, D, Falcone, J, Marsh, R, Moore, N, Tye, N, Seeman, P, Wong, D (1996a) Radioreceptor binding profile of the atypical antipsychotic olanzapine. *Neuropsychopharmacology*, 14(2): 87-96.
- Bymaster, F, Hemrick-Luecke, S, Perry, K, Fuller, R (1996b) Neurochemical evidence for antagonism by olanzapine of dopamine, serotonin, alpha1-adrenergic and muscarinic receptors in vivo in rats. *Psychopharmacology*, 124(1): 87-94.
- Bymaster, FP, Felder, CC (2002) Role of the cholinergic muscarinic system in bipolar disorder and related mechanism of action of antipsychotic agents. *Molecular Psychiatry*, 7(1).
- Bymaster, FP, Nelson, DL, DeLapp, NW, Falcone, JF, Eckols, K, Truex, LL, Foreman, MM, Lucaites, VL, Calligaro, DO (1999) Antagonism by olanzapine of dopamine D1,

- serotonin₂, muscarinic, histamine H₁ and [α]₁-adrenergic receptors in vitro. *Schizophrenia Research*, 37(1): 107-122.
- Carlsson, A, Lindqvist, M (1963) Effect of chlorpromazine and haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta Pharmacologica et Toxicologica*, 20: 140-144.
- Caro, JF, Kolaczynski, JW, Nyce, MR, Ohannesian, JP, Opentanova, I, Goldman, WH, Lynn, RB, Zhang, P-L, Sinha, MK, Considine, RV (1996) Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *The Lancet*, 348(9021): 159-161.
- Caulfield, MP, Birdsall, NJM (1998) International Union of Pharmacology. XVII. Classification of Muscarinic Acetylcholine Receptors. *Pharmacological Reviews*, 50(2): 279-290.
- Cavuto, P, Wittert, GA (2009) The role of the endocannabinoid system in the regulation of energy expenditure. *Best Practice & Research Clinical Endocrinology & Metabolism*, 23(1): 79-86.
- Centorrino, F, Eakin, M, Bahk, W-M, Kelleher, JP, Goren, J, Salvatore, P, Egli, S, Baldessarini, RJ (2002) Inpatient Antipsychotic Drug Use in 1998, 1993, and 1989. *American Journal of Psychiatry*, 159(11): 1932-1935.
- Chaudhri, OB, Small, CJ, Bloom, SR (2005) The gastrointestinal tract and the regulation of appetite. *Drug Discovery Today: Disease Mechanisms*, 2(3): 289-294.
- Chee, MJS, Myers, MG, Jr., Price, CJ, Colmers, WF (2010) Neuropeptide Y Suppresses Anorexigenic Output from the Ventromedial Nucleus of the Hypothalamus. *Journal of Neuroscience*, 30(9): 3380-3390.
- Chintoh, AF, Mann, SW, Lam, L, Giacca, A, Fletcher, P, Nobrega, J, Remington, G (2009) Insulin resistance and secretion in vivo: Effects of different antipsychotics in an animal model. *Schizophrenia Research*, 108(1-3): 127-133.
- Chintoh, AF, Mann, SW, Lam, L, Lam, C, Cohn, TA, Fletcher, PJ, Nobrega, JN, Giacca, A, Remington, G (2008a) Insulin resistance and decreased glucose-stimulated insulin secretion after acute olanzapine administration. *Journal of Clinical Psychopharmacology*, 28(5): 494-499.
- Chintoh, AF, Mann, SW, Lam, TKT, Giacca, A, Remington, G (2008b) Insulin resistance following continuous, chronic olanzapine treatment: An animal model. *Schizophrenia Research*, 104(1-3): 23-30.
- Chiu, C-C, Chen, C-H, Chen, B-Y, Yu, S-H, Lu, M-L (2010) The time-dependent change of insulin secretion in schizophrenic patients treated with olanzapine. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 34(6): 866-870.
- Chiu, C, Chen, K, Liu, H, Lu, M (2006) The Early Effect of Olanzapine and Risperidone on Insulin Secretion in Atypical-naïve Schizophrenic Patients. *Journal of Clinical Psychopharmacology*, 26(5): 504-507.
- Choi, S, DiSilvio, B, Unangst, J, Fernstrom, JD (2007) Effect of chronic infusion of olanzapine and clozapine on food intake and body weight gain in male and female rats. *Life Sciences*, 81(12): 1024-1030.
- Coccurello, R, Brina, D, Caprioli, A, Conti, R, Ghirardi, O, Schepis, F, Moles, A (2009) 30 Days of Continuous Olanzapine Infusion Determines Energy Imbalance, Glucose Intolerance, Insulin Resistance, and Dyslipidemia in Mice. *Journal of Clinical Psychopharmacology*, 29(6): 576-583.
- Coccurello, R, Caprioli, A, Conti, R, Ghirardi, O, Borsini, F, Carminati, P, Moles, A (2008) Olanzapine (LY170053, 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5]benzodiazepine), but not the novel atypical antipsychotic ST2472 (9-piperazin-1-ylpyrrolo[2,1-b][1,3]benzothiazepine), chronic administration induces weight gain,

- hyperphagia, and metabolic dysregulation in mice. *Journal of Pharmacology & Experimental Therapeutics*, 326(3): 905-911.
- Coccurello, R, Moles, A (2010) Potential mechanisms of atypical antipsychotic-induced metabolic derangement: Clues for understanding obesity and novel drug design. *Pharmacology & Therapeutics*, 127(3): 210-251.
- Cone, R (2005) Anatomy and regulation of the central melanocortin system. *Nature Neuroscience*, 8(5): 571-578.
- Cone, RD, Cowley, MA, Butler, AA, Fan, W, Marks, DL, Low, MJ (2001) The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. *International Journal of Obesity and Related Disorders*, 25(S5): S63.
- Cooper, G, Pickavance, L, Wilding, J, Halford, J, Goudie, A (2005) A parametric analysis of olanzapine-induced weight gain in female rats. *Psychopharmacology*, 181: 80-89.
- Cooper, GD, Harrold, JA, Halford, JCG, Goudie, AJ (2008a) Chronic clozapine treatment in female rats does not induce weight gain or metabolic abnormalities but enhances adiposity: Implications for animal models of antipsychotic-induced weight gain. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 32(2): 428-436.
- Cooper, MS, Reeve Jr, JR, Abdalla, MO, Moyer, L, Raboin, SJ, Green, GM, Sayegh, AI (2008b) Cholecystokinin-33 is more effective than cholecystokinin-8 in inhibiting food intake and in stimulating the myenteric plexus and dorsal vagal complex. *Brain Research*, 1205: 27-35.
- Cope, MB, Li, X, Jumbo-Lucioni, P, DiCostanzo, CA, Jamison, WG, Kesterson, RA, Allison, DB, Nagy, TR (2009) Risperidone alters food intake, core body temperature, and locomotor activity in mice. *Physiology and Behavior*, 96(3): 457-463.
- Cope, MB, Nagy, TR, Fernandez, JR, Geary, N, Casey, DE, Allison, DB (2005) Antipsychotic drug-induced weight gain: development of an animal model. *International Journal of Obesity and Related Metabolic Disorders*, 29(6): 607-614.
- Coppari, R, Ichinose, M, Lee, CE, Pullen, AE, Kenny, CD, McGovern, RA, Tang, V, Liu, SM, Ludwig, T, Chua Jr, SC, Lowell, BB, Elmquist, JK (2005) The hypothalamic arcuate nucleus: A key site for mediating leptin's effects on glucose homeostasis and locomotor activity. *Cell Metabolism*, 1(1): 63-72.
- Correll, CU, Malhotra, AK (2004) Pharmacogenetics of antipsychotic-induced weight gain. *Psychopharmacology*, 174(4): 477-489.
- Costoli, T, Sgoifo, A, Stilli, D, Flugge, G, Adriani, W, Laviola, G, Fuchs, E, Pedrazzini, T, Musso, E (2005) Behavioural, neural and cardiovascular adaptations in mice lacking the NPY Y1 receptor. *Neuroscience and Biobehavioral Reviews*, 29(1): 113-123.
- Cota, D, Marsicano, G, Lutz, B, Vicennati, V, Stalla, GK, Pasquali, R, Pagotto, U (2003a) Endogenous cannabinoid system as a modulator of food intake. *International Journal of Obesity*, 27(3): 289-301.
- Cota, D, Marsicano, G, Tschöp, M, Glubler, Y, Flachskamm, C, Schubert, M, Auer, D, Yassouridis, A, Thone-Reineke, C, Ortmann, S, Tomassoni, F, Cervino, C, Nisoli, E, Linthorst, A, Pasquali, R, Lutz, B, Stalla, GK, Pagotto, U (2003b) The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *Journal of Clinical Investigation*, 112(3): 423-431.
- Cowley, MA, Smart, JL, Rubinstein, M, Cerdan, MG, al, e (2001) Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature*, 411(6836): 480.
- Cowley, MA, Smith, RG, Diano, S, Tschöp, M, Pronchuk, N, Grove, KL, Strasburger, CJ, Bidlingmaier, M, Esterman, M, Heiman, ML, Garcia-Segura, LM, Nillni, EA, Mendez, P, Low, MJ, Sotonyi, P, Friedman, JM, Liu, H, Pinto, S, Colmers, WF, Cone, RD, Horvath, TL (2003) The Distribution and Mechanism of Action of Ghrelin in the CNS Demonstrates a Novel Hypothalamic Circuit Regulating Energy Homeostasis. *Neuron*, 37(4): 649-661.

- Crook, JM, Tomaskovic-Crook, E, Copolov, DL, Dean, B (2001) Low Muscarinic Receptor Binding in Prefrontal Cortex From Subjects With Schizophrenia: A Study of Brodmann's Areas 8, 9, 10, and 46 and the Effects of Neuroleptic Drug Treatment. *American Journal of Psychiatry*, 158(6): 918-925.
- Csiffáry, A, Görcs, TJ, Palkovits, M (1990) Neuropeptide Y innervation of ACTH-immunoreactive neurons in the arcuate nucleus of rats: a correlated light and electron microscopic double immunolabeling study. *Brain Research*, 506(2): 215-222.
- Das, UN (2010) Vagus nerve stimulation as a strategy to prevent and manage metabolic syndrome. *Medical Hypotheses*, 76(3): 429-433.
- Dautzenberg, FM, Neysari, S (2005) Irreversible binding kinetics of neuropeptide Y ligands to Y2 but not to Y1 and Y5 receptors. *Pharmacology*, 75(1): 21-29.
- Davoodi, N, Kalinichev, M, Korneev, S, Clifton, P (2009) Hyperphagia and increased meal size are responsible for weight gain in rats treated sub-chronically with olanzapine. *Psychopharmacology*, 203(4): 693-702.
- De Hert, M, Dobbelaere, M, Sheridan, EM, Cohen, D, Correll, CU (2011) Metabolic and endocrine adverse effects of second-generation antipsychotics in children and adolescents: A systematic review of randomized, placebo controlled trials and guidelines for clinical practice. *European Psychiatry*, 26(3): 144-158.
- de Leon, J, Verghese, C, Josiassen, R, Simpson, G (1994) Polydipsia and water intoxication in psychiatric patients: a review of the epidemiological literature. *Biological Psychiatry*, 35: 519-530.
- de Meijer, VE, Le, HD, Meisel, JA, Puder, M (2010) Repetitive orogastric gavage affects the phenotype of diet-induced obese mice. *Physiology and Behavior*, 100(4): 387-393.
- Dean, B, Gray, L, Keriakous, D, Scarr, E (2004) A comparison of M1 and M4 muscarinic receptors in the thalamus from control subjects and subjects with schizophrenia. *Thalamus & Related Systems*, 2(4): 287-295.
- Deng, C, Han, M, Huang, X (2007a) No changes in densities of cannabinoid receptors in the superior temporal gyrus in schizophrenia. *Neuroscience Bulletin*, 23(6): 341-347.
- Deng, C, Huang, X-F (2005) Decreased density of muscarinic receptors in the superior temporal gyrus in schizophrenia. *Journal of Neuroscience Research*, 81(6): 883-890.
- Deng, C, Weston-Green, K, Huang, X-F (2010) The role of histaminergic H1 and H3 receptors in food intake: A mechanism for atypical antipsychotic-induced weight gain? *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 34(1): 1-4.
- Deng, C, Weston-Green, KL, Han, M, Huang, X-F (2007b) Olanzapine treatment decreases the density of muscarinic M2 receptors in the dorsal vagal complex of rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 31(4): 915-920.
- Derbenev, AV, Stuart, TC, Smith, BN (2004) Cannabinoids suppress synaptic input to neurones of the rat dorsal motor nucleus of the vagus nerve. *Journal of Physiology*, 559(3): 923-938.
- Di Marzo, V, Goparaju, SK, Wang, L, Liu, J, Batkai, S, Jarai, Z, Fezza, F, Miura, GI, Palmiter, RD, Sugiura, T, Kunos, G (2001) Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature*, 410(6830): 822-825.
- Dockray, GJ (2009) The versatility of the vagus. *Physiology and Behavior*, 97(5): 531-536.
- du Bois, TM, Huang, X-F, Deng, C (2008) Perinatal administration of PCP alters adult behaviour in female Sprague-Dawley rats. *Behavioural Brain Research*, 188(2): 416-419.
- Dumont, Y, Fournier, A, St-Pierre, S, Quirion, R (1996) Autoradiographic distribution of [125I]Leu31,Pro34]PYY and [125I]PYY3-36 binding sites in the rat brain evaluated with two newly developed Y1 and Y2 receptor radioligands. *Synapse*, 22(2): 139-158.

- Duttaroy, A, Zimlik, CL, Gautam, D, Cui, Y, Mears, D, Wess, J (2004) Muscarinic stimulation of pancreatic insulin and glucagon release is abolished in M3 muscarinic acetylcholine receptor-deficient mice. *Diabetes*, 53(7): 1714(1717).
- Edelsbrunner, ME, Herzog, H, Holzer, P (2009a) Evidence from knockout mice that peptide YY and neuropeptide Y enforce murine locomotion, exploration and ingestive behaviour in a circadian cycle- and gender-dependent manner. *Behavioural Brain Research*, 203(1): 97-107.
- Edelsbrunner, ME, Painsipp, E, Herzog, H, Holzer, P (2009b) Evidence from knockout mice for distinct implications of neuropeptide-Y Y2 and Y4 receptors in the circadian control of locomotion, exploration, water and food intake. *Neuropeptides*, 43(6): 491-497.
- Eder, U, Mangweth, B, Ebenbichler, C, Weiss, E, Hofer, A, Hummer, M, Kemmler, G, Lechleitner, M, Fleischhacker, WW (2001) Association of olanzapine-induced weight gain with an increase in body fat. *The American Journal of Psychiatry*, 158(10): 1719.
- Elmqvist, JK, Marcus, JN (2003) Rethinking the central causes of diabetes. *Nature Medicine*, 9(6): 645-647.
- Erickson, JC, Clegg, KE, Palmiter, RD (1996) Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature*, 381(6581): 415-418.
- Esclapez, M, Tillakaratne, NJ, Kaufman, DL, Tobin, AJ, Houser, CR (1994) Comparative localization of two forms of glutamic acid decarboxylase and their mRNAs in rat brain supports the concept of functional differences between the forms. *The Journal of Neuroscience*, 14(3): 1834-1855.
- Esen-Danaci, A, Sarandöl, A, Taneli, F, Yurtsever, F, Özlen, N (2008) Effects of second generation antipsychotics on leptin and ghrelin. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 32(6): 1434-1438.
- Fakhoury, WKH, Wright, D, Wallace, M (2001) Prevalence and extent of distress of adverse effects of antipsychotics among callers to a United Kingdom National Mental Health Helpline. *International Clinical Psychopharmacology*, 16(3): 153-162.
- Fan, W, Ellacott, KLJ, Halatchev, IG, Takahashi, K, Yu, P, Cone, RD (2004) Cholecystokinin-mediated suppression of feeding involves the brainstem melanocortin system. *Nature Neuroscience*, 7(4): 335-336.
- Farde, L, Nordstrom, A, Wiesel, F, Pauli, S, Halldin, C, G, S (1992) Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine: relation to extrapyramidal side effects. *Archives of General Psychiatry*, 49: 538-544.
- Faulconbridge, LF, Grill, HJ, Kaplan, JM, Daniels, D (2008) Caudal brainstem delivery of ghrelin induces fos expression in the nucleus of the solitary tract, but not in the arcuate or paraventricular nuclei of the hypothalamus. *Brain Research*, 1218(C): 151-157.
- Feldblum, S, Erlander, MG, Tobin, AJ (1993) Different distributions of GAD65 and GAD67 mRNAs suggest that the two glutamate decarboxylases play distinctive functional roles. *Journal of Neuroscience Research*, 34(6): 689-706.
- Fell, M, Marshall, K, Williams, J, Neill, J (2004) Effects of the atypical antipsychotic olanzapine on reproductive function and weight gain in female rats. *Journal of Psychopharmacology*, 18(2): 149-155.
- Ferno, J, Varela, L, Skrede, S, Vazquez, MJ, Nogueiras, R, Dieguez, C, Vidal-Puig, A, Steen, VM, Lopez, M (2011) Olanzapine-Induced Hyperphagia and Weight Gain Associate with Orexigenic Hypothalamic Neuropeptide Signaling without Concomitant AMPK Phosphorylation. *PLoS ONE*, 6(6): e20571.
- Fetissov, SO, Kopp, J, Hökfelt, T (2004) Distribution of NPY receptors in the hypothalamus. *Neuropeptides*, 38(4): 175-188.

- Fitzgerald, P, Scaffidi, A, Morris, M, de Castella, A, Kulkarni, J (2003) The relationship of changes in leptin, neuropeptide Y and reproductive hormones to antipsychotic induced weight gain. *Human Psychopharmacology: Clinical and Experimental*, 18(7): 551-557.
- Frenchman, IB (2005) Atypical antipsychotics for nursing home patients: a retrospective chart review. *Drugs & Aging*, 22(3): 257-264.
- Friedman, JM, Halaas, JL (1998) Leptin and the regulation of body weight in mammals. *Nature*, 395(6704): 763-770.
- Frohman, LA, Bernardis, LL, Schnatz, JD, Burek, L (1969) Plasma insulin and triglyceride levels after hypothalamic lesions in weanling rats. *American Journal of Physiology*, 216: 1496-1501.
- Fukudome, Y, Ohno-Shosaku, T, Matsui, M, Omori, Y, Fukaya, M, Tsubokawa, H, Taketo, MM, Watanabe, M, Manabe, T, Kano, M (2004) Two distinct classes of muscarinic action on hippocampal inhibitory synapses: M2-mediated direct suppression and M1/M3-mediated indirect suppression through endocannabinoid signalling. *European Journal of Neuroscience*, 19(10): 2682-2692.
- Gamber, KM, Macarthur, H, Westfall, TC (2005) Cannabinoids augment the release of neuropeptide Y in the rat hypothalamus. *Neuropharmacology*, 49(5): 646-652.
- Gao, X-M, Cooper, T, Suckow, RF, Tamminga, CA (2005) Multidose Risperidone Treatment Evaluated in a Rodent Model of Tardive Dyskinesia. *Neuropsychopharmacology*, 31(9): 1864-1868.
- Gautam, D, de Azua, IR, Li, JH, Guettier, J-M, Heard, T, Cui, Y, Lu, H, Jou, W, Gavrilova, O, Zawulich, WS, Wess, J (2010) Beneficial Metabolic Effects Caused by Persistent Activation of β -Cell M3 Muscarinic Acetylcholine Receptors in Transgenic Mice. *Endocrinology*, 151(11): 5185-5194.
- Gautam, D, Gavrilova, O, Jeon, J, Pack, S, Jou, W, Cui, Y, Li, JH, Wess, J (2006a) Beneficial metabolic effects of M3 muscarinic acetylcholine receptor deficiency. *Cell Metabolism*, 4(5): 363-375.
- Gautam, D, Han, S-J, Hamdan, FF, Jeon, J, Li, B, Li, JH, Cui, Y, Mears, D, Lu, H, Deng, C, Heard, T, Wess, J (2006b) A critical role for β cell M3 muscarinic acetylcholine receptors in regulating insulin release and blood glucose homeostasis in vivo. *Cell Metabolism*, 3(6): 449-461.
- Gautam, D, Jeon, J, Li, JH, Han, S-J, Hamdan, FF, Cui, Y, Lu, H, Deng, C, Gavrilova, O, Wess, J (2008) Metabolic Roles of the M3 Muscarinic Acetylcholine Receptor Studied with M3 Receptor Mutant Mice: A Review. *Journal of Receptors and Signal Transduction*, 28(1-2): 93-108.
- Geary, N (2004) Endocrine controls of eating: CCK, leptin, and ghrelin. *Physiology and Behavior*, 81(5): 719-733.
- Gebhardt, S, Haberhausen, M, Heinzel-Gutenbrunner, M, Gebhardt, N, Remschmidt, H, Krieg, J-C, Hebebrand, J, Theisen, FM (2009) Antipsychotic-induced body weight gain: Predictors and a systematic categorization of the long-term weight course. *Journal of Psychiatric Research*, 43(6): 620-626.
- Gibbons, AS, Scarr, E, McLean, C, Sundram, S, Dean, B (2009) Decreased muscarinic receptor binding in the frontal cortex of bipolar disorder and major depressive disorder subjects. *Journal of Affective Disorders*, 116(3): 184-191.
- Gilon, P, Henquin, J-C (2001) Mechanisms and Physiological Significance of the Cholinergic Control of Pancreatic β -Cell Function. *Endocrine Reviews*, 22(5): 565-604.
- Glass, M, Faull, RLM, Dragunow, M (1997) Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience*, 77(2): 299-318.

- Goldman, MB, Hussain, N (2004) Absence of effect of olanzapine on primary polydipsia: results of a double-blind, randomized study. *Journal of Clinical Psychopharmacology*, 24(6): 678-680.
- Gomez, R, Navarro, M, Ferrer, B, Trigo, JM, Bilbao, A, Del Arco, I, Cippitelli, A, Nava, F, Piomelli, D, Rodriguez de Fonseca, F (2002) A Peripheral Mechanism for CB1 Cannabinoid Receptor-Dependent Modulation of Feeding. *Journal of Neuroscience*, 22(21): 9612-9617.
- Gong, J-P, Onaivi, ES, Ishiguro, H, Liu, Q-R, Tagliaferro, PA, Brusco, A, Uhl, GR (2006) Cannabinoid CB2 receptors: Immunohistochemical localization in rat brain. *Brain Research*, 1071(1): 10-23.
- Goossens, GH (2008) The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. *Physiology and Behavior*, 94(2): 206-218.
- Gothelf, D, Falk, B, Singer, P, Kairi, M, Phillip, M, Zigel, L, Poraz, I, Frishman, S, Constantini, N, Zalsman, G, Weizman, A, Apter, A (2002) Weight Gain Associated With Increased Food Intake and Low Habitual Activity Levels in Male Adolescent Schizophrenic Inpatients Treated With Olanzapine. *American Journal of Psychiatry*, 159(6): 1055-1057.
- Goudie, A, Smith, J, Halford, J (2002) Characterization of olanzapine-induced weight gain in rats. *Journal of Psychopharmacology*, 16(4): 291-296.
- Graham, K, A, Perkins, D, O, Edwards, L, J, Barrier, R, C Jr, et al. (2005) Effect of Olanzapine on Body Composition and Energy Expenditure in Adults With First-Episode Psychosis. *The American Journal of Psychiatry*, 162(1): 118.
- Gray, TS, O'Donohue, TL, Magnuson, DJ (1986) Neuropeptide Y innervation of amygdaloid and hypothalamic neurons that project to the dorsal vagal complex in rat. *Peptides*, 7(2): 341-349.
- Gromada, J, Hughes, TE (2006) Ringing the dinner bell for insulin: Muscarinic M3 receptor activity in the control of pancreatic [beta] cell function. *Cell Metabolism*, 3(6): 390-392.
- Gropp, E, Shanabrough, M, Borok, E, Xu, AW, Janoschek, R, Buch, T, Plum, L, Balthasar, N, Hampel, B, Waisman, A, Barsh, GS, Horvath, TL, Bruning, JC (2005) Agouti-related peptide-expressing neurons are mandatory for feeding. *Nature Neuroscience*, 8(10): 1289-1291.
- Guan, X-M, Yu, H, Van der Ploeg, LHT (1998) Evidence of altered hypothalamic pro-opiomelanocortin/ neuropeptide Y mRNA expression in tubby mice. *Molecular Brain Research*, 59(2): 273-279.
- Guillod-Maximin, E, Lorsignol, A, Alquier, T, Pénicaud, L (2004) Acute intracarotid glucose injection towards the brain induces specific c-fos activation in hypothalamic nuclei: involvement of astrocytes in cerebral glucose-sensing in rats. *Journal of Neuroendocrinology*, 16(5): 464-471.
- Gutzwiller, J-P, Drewe, J, Ketterer, S, Hildebrand, P, Krautheim, A, Beglinger, C (2000) Interaction between CCK and a preload on reduction of food intake is mediated by CCK-A receptors in humans. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 279(1): R189-195.
- Hakko, H, Komulainen, MT, Koponen, H, Saari, K, Laitinen, J, Järvelin, M-R, Lindeman, S (2006) Are females at special risk of obesity if they become psychotic? The longitudinal Northern Finland 1966 Birth Cohort Study. *Schizophrenia Research*, 84(1): 15-19.
- Halaas, JL, Gajiwala, KS, Maffei, M, Cohen, SL, Chait, BT, Rabinowitz, D, Lallone, RL, Burley, SK, Friedman, JM (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. *Science*, 269(5223): 543-546.
- Hales, CN, Kennedy, GC (1964) Plasma glucose, nonesterified fatty acid and insulin concentrations in hypothalamic-hyperphagia rats. *Biochemical Journal*, 90: 620-624.

- Han, M, Deng, C, Burne, THJ, Newell, KA, Huang, X-F (2008a) Short- and long-term effects of antipsychotic drug treatment on weight gain and H1 receptor expression. *Psychoneuroendocrinology*, 33(5): 569-580.
- Han, M, Huang, X-F, Deng, C (2009) Aripiprazole differentially affects mesolimbic and nigrostriatal dopaminergic transmission: implications for long-term drug efficacy and low extrapyramidal side-effects. *The International Journal of Neuropsychopharmacology*, 12(7): 941-952.
- Han, M, Newell, K, Zavitsanou, K, Deng, C, Huang, X-F (2008b) Effects of antipsychotic medication on muscarinic M1 receptor mRNA expression in the rat brain. *Journal of Neuroscience Research*, 86(2): 457-464.
- Haro, JM, Novick, D, Suarez, D, Roca, M (2009) Antipsychotic treatment discontinuation in previously untreated patients with schizophrenia: 36-month results from the SOHO study. *Journal of Psychiatric Research*, 43(3): 265-273.
- Harrold, J, Williams, G (2003) The cannabinoid system: a role in both the homeostatic and hedonic control of eating? *British Journal of Nutrition*, 90: 729-734.
- Hartfield, A, Moore, N, Clifton, P (2003) Effects of clozapine, olanzapine and haloperidol on the microstructure of ingestive behaviour in the rat. *Psychopharmacology*, 167(2): 115.
- Heilig, M, Murison, R (1987) Intracerebroventricular neuropeptide Y suppresses open field and home cage activity in the rat. *Regulatory Peptides*, 19(3-4): 221-231.
- Heilig, M, Vecsei, L, Widerlöv, E (1989) Opposite effects of centrally administered neuropeptide Y (NPY) on locomotor activity of spontaneously hypertensive (SH) and normal rats. *Acta Physiologica Scandinavica*, 137(2): 243-248.
- Heilig, M, Wahlestedt, C, Widerlöv, E (1988) Neuropeptide Y (NPY)-induced suppression of activity in the rat: evidence for NPY receptor heterogeneity and for interaction with [alpha]-adrenoceptors. *European Journal of Pharmacology*, 157(2-3): 205-213.
- Heisler, LK, Cowley, MA, Tecott, LH, Fan, W, Low, MJ, Smart, JL, Rubinstein, M, Tatro, JB, Marcus, JN, Holstege, H, Lee, CE, Cone, RD, Elmquist, JK (2002) Activation of Central Melanocortin Pathways by Fenfluramine. *Science*, 297(5581): 609-611.
- Hentges, ST, Low, MJ, Williams, JT (2005) Differential Regulation of Synaptic Inputs by Constitutively Released Endocannabinoids and Exogenous Cannabinoids. *Journal of Neuroscience*, 25(42): 9746-9751.
- Hentges, ST, Nishiyama, M, Overstreet, LS, Stenzel-Poore, M, Williams, JT, Low, MJ (2004) GABA Release from Proopiomelanocortin Neurons. *The Journal of Neuroscience*, 24(7): 1578-1583.
- Heymsfield, SB, Greenberg, AS, Fujioka, K, Dixon, RM, Kushner, R, Hunt, T, Lubina, JA, Patane, J, Self, B, Hunt, P, McCamish, M (1999) Recombinant Leptin for Weight Loss in Obese and Lean Adults: A Randomized, Controlled, Dose-Escalation Trial. *Journal of the American Medical Association*, 282(16): 1568-1575.
- Hiejima, H, Nishi, Y, Hosoda, H, Yoh, J, Mifune, H, Satou, M, Sugimoto, H, Chiba, S, Kawahara, Y, Tanaka, E, Yoshimatsu, H, Uchimura, N, Kangawa, K, Kojima, M (2009) Regional distribution and the dynamics of n-decanoyl ghrelin, another acyl-form of ghrelin, upon fasting in rodents. *Regulatory Peptides*, 156(1-3): 47-56.
- Hippius, H (1989) The history of clozapine. *Psychopharmacology*, 99(Suppl.): S3-S5.
- Ho, J, Cox, JM, Wagner, EJ (2007) Cannabinoid-induced hyperphagia: Correlation with inhibition of proopiomelanocortin neurons? *Physiology and Behavior*, 92(3): 507-519.
- Hollingworth, SA, Siskind, DJ, Nissen, LM, Robinson, M, Hall, WD (2010) Patterns of antipsychotic medication use in Australia 2002-2007. *Australian and New Zealand Journal of Psychiatry*, 44(4): 372-377.
- Hollopeter, G, Erickson, JC, Seeley, RJ, Marsh, DJ, Palmiter, RD (1998) Response of neuropeptide Y-deficient mice to feeding effectors. *Regulatory Peptides*, 75-76: 383-389.

- Horvath, TL, Bechmann, I, Naftolin, F, Kalra, SP, Leranath, C (1997) Heterogeneity in the neuropeptide Y-containing neurons of the rat arcuate nucleus: GABAergic and non-GABAergic subpopulations. *Brain Research*, 756(1-2): 283-286.
- Hosojima, H, Togo, T, Odawara, T, Hasegawa, K, Miura, S, Kato, Y, Kanai, A, Kase, A, Uchikado, H, Hirayasu, Y (2006) Early effects of olanzapine on serum levels of ghrelin, adiponectin and leptin in patients with schizophrenia. *Journal of Psychopharmacology*, 20(1): 75-79.
- Hou, Z, Miao, Y, Gao, L, Pan, H, Zhu, S (2006) Ghrelin-containing neuron in cerebral cortex and hypothalamus linked with the DVC of brainstem in rat. *Regulatory Peptides*, 134(2-3): 126-131.
- Houseknecht, KL, Robertson, AS, Zavadoski, W, Gibbs, EM, Johnson, DE, Rollema, H (2006) Acute Effects of Atypical Antipsychotics on Whole-Body Insulin Resistance in Rats: Implications for Adverse Metabolic Effects. *Neuropsychopharmacology*, 32(2): 289-297.
- Howes, OD, Kapur, S (2009) The Dopamine Hypothesis of Schizophrenia: Version III "The Final Common Pathway". *Schizophrenia Bulletin*, 35(3): 549-562.
- Howlett, AC, Breivogel, CS, Childers, SR, Deadwyler, SA, Hampson, RE, Porrino, LJ (2004) Cannabinoid physiology and pharmacology: 30 years of progress. *Neuropharmacology*, 47(Supplement 1): 345-358.
- Huang-Brown, KM, Guhad, FA (2002) Chocolate, an effective means of oral drug delivery in rats. *Lab Animal*, 31(10): 34-36.
- Huang, X-F, Han, M, Huang, X, Zavitsanou, K, Deng, C (2006a) Olanzapine differentially affects 5-HT_{2A} and 2C receptor mRNA expression in the rat brain. *Behavioural Brain Research*, 171(2): 355-362.
- Huang, X-F, Han, M, South, T, Storlien, L (2003) Altered levels of POMC, AgRP and MC4-R mRNA expression in the hypothalamus and other parts of the limbic system of mice prone or resistant to chronic high-energy diet-induced obesity. *Brain Research*, 992(1): 9-19.
- Huang, XF, Deng, C, Zavitsanou, K (2006b) Neuropeptide Y mRNA expression levels following chronic olanzapine, clozapine and haloperidol administration in rats. *Neuropeptides*, 40(3): 213-219.
- Huo, L, Gamber, K, Greeley, S, Silva, J, Huntoon, N, Leng, X-H, Bjørbaek, C (2009) Leptin-Dependent Control of Glucose Balance and Locomotor Activity by POMC Neurons. *Cell Metabolism*, 9(6): 537-547.
- Idänpään-Heikkilä, J, Alhava, E, Olkinuora, M, Palva, IP (1977) Agranulocytosis during treatment with clozapine. *European Journal of Clinical Pharmacology*, 11(3): 193-198.
- Istvan, Katona, I, Sperlagh, B, Sik, A, Kafalvi, A, Vizi, E, Mackie, K, Freund, T (1999) Presynaptically Located CB1 Cannabinoid Receptors Regulate GABA Release from Axon Terminals of Specific Hippocampal Interneurons. *The Journal of Neuroscience*, 19(11): 4544-4558.
- Jin, H, Meyer, JM, Mudaliar, S, Jeste, DV (2008) Impact of atypical antipsychotic therapy on leptin, ghrelin, and adiponectin. *Schizophrenia Research*, 100(1-3): 70-85.
- Jindal, R, Keshavan, M (2006) Critical Role of M3 Muscarinic Receptor in Insulin Secretion: Implications for Psychopharmacology. *Journal of Clinical Psychopharmacology*, 26(5): 449-450.
- Johnson, DE, Yamazaki, H, Ward, KM, Schmidt, AW, Lebel, WS, Treadway, JL, Gibbs, EM, Zawalich, WS, Rollema, H (2005) Inhibitory Effects of Antipsychotics on Carbachol-Enhanced Insulin Secretion From Perfused Rat Islets: Role of Muscarinic Antagonism in Antipsychotic-Induced Diabetes and Hyperglycemia. *Diabetes*, 54(5): 1552-1558.
- Kalinichev, M, Rourke, C, Daniels, A, Grizzle, M, Britt, C, Ignar, D, Jones, D (2005) Characterisation of olanzapine-induced weight gain and effect of aripiprazole vs

- olanzapine on body weight and prolactin secretion in female rats. *Psychopharmacology*, 182: 220-231.
- Kalra, SP (2008) Disruption in the leptin-NPY link underlies the pandemic of diabetes and metabolic syndrome: New therapeutic approaches. *Nutrition*, 24(9): 820-826.
- Kalra, SP, Kalra, PS (2003) Neuropeptide Y: a physiological orexigen modulated by the feedback action of ghrelin and leptin. *Endocrine*, 22(1): 49-56.
- Kane, J, Honigfeld, G, Singer, J, Meltzer, H (1988) Clozapine for the Treatment-Resistant Schizophrenic: A Double-blind Comparison With Chlorpromazine. *Archives of General Psychiatry*, 45(9): 789-796.
- Kapur, S (1998) A new framework for investigating antipsychotic action in humans: lessons from PET imaging. *Molecular Psychiatry*, 3: 135-140.
- Kapur, S, Remington, G (2001) Atypical Antipsychotics: New Directions and New Challenges in the Treatment of Schizophrenia. *Annual Review of Medicine*, 52(1): 503-517.
- Kapur, S, VanderSpek, SC, Brownlee, BA, Nobrega, J (2003) Antipsychotic Dosing in Preclinical Models Is Often Unrepresentative of the Clinical Condition: A Suggested Solution Based on in Vivo Occupancy. *Journal of Pharmacology and Experimental Therapeutics*, 305(2): 625-631.
- Kapur, S, Wadenberg, ML, Remington, G (2000) Are animal studies of antipsychotics appropriately dosed? Lessons from the bedside to the bench. *Canadian Journal Of Psychiatry. Revue Canadienne De Psychiatrie*, 45(3): 241-246.
- Karl, T, Duffy, L, Herzog, H (2008) Behavioural profile of a new mouse model for NPY deficiency. *European Journal of Neuroscience*, 28(1): 173-180.
- Karl, T, Duffy, L, O'Brien, E, Matsumoto, I, Dedova, I (2006) Behavioural effects of chronic haloperidol and risperidone treatment in rats. *Behavioural Brain Research*, 171(2): 286-294.
- Kaufman, DL, Houser, CR, Tobin, AJ (1991) Two Forms of the γ -Aminobutyric Acid Synthetic Enzyme Glutamate Decarboxylase Have Distinct Intraneuronal Distributions and Cofactor Interactions. *Journal of Neurochemistry*, 56(2): 720-723.
- Kawamura, Y, Fukaya, M, Maejima, T, Yoshida, T, Miura, E, Watanabe, M, Ohno-Shosaku, T, Kano, M (2006) The CB1 Cannabinoid Receptor Is the Major Cannabinoid Receptor at Excitatory Presynaptic Sites in the Hippocampus and Cerebellum. *The Journal of Neuroscience*, 26(11): 2991-3001.
- Keefe, RSE, Young, CA, Rock, SL, Purdon, SE, Gold, JM, Breier, A (2006) One-year double-blind study of the neurocognitive efficacy of olanzapine, risperidone, and haloperidol in schizophrenia. *Schizophrenia Research*, 81(1): 1-15.
- Kelly, DL, Conley, RR, Tamminga, CA (1999) Differential olanzapine plasma concentrations by sex in a fixed-dose study. *Schizophrenia Research*, 40(2): 101-104.
- Kern, RS, Glynn, SM, Horan, WP, Marder, SR (2009) Psychosocial Treatments to Promote Functional Recovery in Schizophrenia. *Schizophrenia Bulletin*, 35(2): 347-361.
- Kim, B-J, Sohn, J-W, Park, C-S, Hahn, G-H, Koo, J, Noh, Y-D, Lee, C-S (2008) Body weight and plasma levels of ghrelin and leptin during treatment with olanzapine. *Journal of Korean Medical Science*, 23(4): 685-690.
- Kim, E-M, O'Hare, E, Grace, MK, Welch, CC, Billington, CJ, Levine, AS (2000) ARC POMC mRNA and PVN [alpha]-MSH are lower in obese relative to lean Zucker rats. *Brain Research*, 862(1-2): 11-16.
- Kim, SF, Huang, AS, Snowman, AM, Teuscher, C, Snyder, SH (2007) Antipsychotic drug-induced weight gain mediated by histamine H1 receptor-linked activation of hypothalamic AMP-kinase. *Proceedings of the National Academy of Sciences*, 104(9): 3456-3459.

- Kirk, S, Glazebrook, J, Grayson, B, Neill, J, Reynolds, G (2009) Olanzapine-induced weight gain in the rat: role of 5-HT_{2C} and histamine H₁ receptors. *Psychopharmacology*, 207(1): 119-125.
- Kirk, SL, Cahir, M, Reynolds, GP (2006) Clozapine, but not haloperidol, increases neuropeptide Y neuronal expression in the rat hypothalamus. *Journal of Psychopharmacology*, 20(4): 577-579.
- Kirkham, T (2008) Endocannabinoids and the neurochemistry of gluttony. *Journal of Neuroendocrinology*, 20(9): 1099-1100.
- Kirkham, T, Williams, C, Fezza, F, Di Marzo, V (2002) Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *British Journal of Pharmacology*, 136(4): 550-557.
- Kirkham, TC (2009) Cannabinoids and appetite: Food craving and food pleasure. *International Review of Psychiatry*, 21(2 SPEC. ISS.): 163-171.
- Kluge, M, Schuld, A, Schacht, A, Himmerich, H, Dalal, MA, Wehmeier, PM, Hinze-Selch, D, Kraus, T, Dittmann, RW, Pollmächer, T (2009) Effects of clozapine and olanzapine on cytokine systems are closely linked to weight gain and drug-induced fever. *Psychoneuroendocrinology*, 34(1): 118-128.
- Knauf, C, Cani, PD, Kim, D-H, Iglesias, MA, Chabo, C, Waget, AI, Colom, A, Rastrelli, S, Delzenne, NM, Drucker, DJ, Seeley, RJ, Burcelin, R (2008) Role of Central Nervous System Glucagon-Like Peptide-1 Receptors in Enteric Glucose Sensing. *Diabetes*, 57(10): 2603-2612.
- Kojima, M, Hosoda, H, Date, Y, Nakazato, M, Matsuo, H, Kangawa, K (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, 402(6762): 656-660.
- Kojima, M, Kangawa, K (2005) Ghrelin: Structure and Function. *Physiological Reviews*, 85(2): 495-522.
- Koller, E, Schneider, B, Bennett, K, Dubitsky, G (2001) Clozapine-associated diabetes. *The American Journal of Medicine*, 111(9): 716-723.
- Konturek, SJ, Zabielski, R, Konturek, JW, Czarnecki, J (2003) Neuroendocrinology of the pancreas; role of brain-gut axis in pancreatic secretion. *European Journal of Pharmacology*, 481(1): 1-14.
- Kroeze, WK, Hufeisen, SJ, Popadak, BA, Renock, SM, Steinberg, S, Ernsberger, P, Jayathilake, K, Meltzer, HY, Roth, BL (2003) H₁-Histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. *Neuropsychopharmacology*, 28(3): 519-526.
- Krude, H, Biebermann, H, Luck, W, Horn, R, Brabant, G, Gruters, A (1998) Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. *Nature Genetics*, 19(2): 155-157.
- Kuo, D-Y (2002) Co-administration of dopamine D₁ and D₂ agonists additively decreases daily food intake, body weight and hypothalamic neuropeptide Y level in rats. *Journal of Biomedical Science*, 9(2): 126-132.
- Kuo, D-Y (2006) Hypothalamic neuropeptide Y (NPY) and the attenuation of hyperphagia in streptozotocin diabetic rats treated with dopamine D₁/D₂ agonists. *British Journal of Pharmacology*, 148(5): 640-647.
- Lambert, MT, Copeland, LA, Sampson, N, Duffy, SA (2006) New-onset type-2 diabetes associated with atypical antipsychotic medications. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 30(5): 919-923.
- Lee, H-M, Wang, G, Englander, EW, Kojima, M, Greeley, GH, Jr. (2002) Ghrelin, A New Gastrointestinal Endocrine Peptide that Stimulates Insulin Secretion: Enteric Distribution, Ontogeny, Influence of Endocrine, and Dietary Manipulations. *Endocrinology*, 143(1): 185-190.

- Lee, H, Kwon, K, Alphs, L, Meltzer, H (1991) Effect of clozapine on psychogenic polydipsia in chronic schizophrenia. *Journal of Clinical Psychopharmacology*, 11(3): 222.
- Lee, MD, Clifton, PG (2002) Meal patterns of free feeding rats treated with clozapine, olanzapine, or haloperidol. *Pharmacology Biochemistry and Behavior*, 71(1-2): 147-154.
- Levey, AI, Edmunds, SM, Heilman, CJ, Desmond, TJ, Frey, KA (1994) Localization of muscarinic M3 receptor protein and M3 receptor binding in rat brain. *Neuroscience*, 63(1): 207-221.
- Li, C, Jones, PM, Persaud, SJ (2011) Role of the endocannabinoid system in food intake, energy homeostasis and regulation of the endocrine pancreas. *Pharmacology & Therapeutics*, 129(3): 307-320.
- Li, J, Gautam, D, Han, S, Guettier, J, Cui, Y, Lu, H, Deng, C, O'Hare, J, Jou, W, Gavrilova, O, Buettner, C, Wess, J (2009) Hepatic Muscarinic Acetylcholine Receptors Are Not Critically Involved in Maintaining Glucose Homeostasis in Mice. *Diabetes*, 58(12): 2776.
- Li, Y, Wu, X, Zhu, J, Yan, J, Owyang, C (2003) Hypothalamic regulation of pancreatic secretion is mediated by central cholinergic pathways in the rat. *The Journal of Physiology*, 552(2): 571-587.
- Liddle, RA (2000) Regulation of cholecystokinin secretion in humans. *Journal of Gastroenterology*, 35(3): 181-187.
- Lieberman, JA, Stroup, TS, McEvoy, JP, Swartz, MS, Rosenheck, RA, Perkins, DO, Keefe, RSE, Davis, SM, Davis, CE, Lebowitz, BD, Severe, J, Hsiao, JK (2005) The Clinical Antipsychotic Trials of Intervention Effectiveness of Antipsychotic Drugs in Patients with Chronic Schizophrenia. *New England Journal of Medicine*, 353(12): 1209-1223.
- Lieverse, RJ, Jansen, JB, Masclee, AA, Lamers, CB (1995) Satiety effects of a physiological dose of cholecystokinin in humans. *Gut*, 36(2): 176-179.
- Lin, S, Boey, D, Herzog, H (2004) NPY and Y receptors: lessons from transgenic and knockout models. *Neuropeptides*, 38(4): 189-200.
- Lin, S, Storlien, LH, Huang, X-F (2000) Leptin receptor, NPY, POMC mRNA expression in the diet-induced obese mouse brain. *Brain Research*, 875(1-2): 89-95.
- Ling, LL, Hughes, LF, Caspary, DM (2005) Age-related loss of the GABA synthetic enzyme glutamic acid decarboxylase in rat primary auditory cortex. *Neuroscience*, 132(4): 1103-1113.
- López-Muñoz, F, Alamo, C (2009) The consolidation of neuroleptic therapy: Janssen, the discovery of haloperidol and its introduction into clinical practice. *Brain Research Bulletin*, 79(2): 130-141.
- Lykkegaard, K, Larsen, PJ, Vrang, N, Bock, C, Bock, T, Knudsen, LB (2008) The once-daily human GLP-1 analog, liraglutide, reduces olanzapine-induced weight gain and glucose intolerance. *Schizophrenia Research*, 103(1-3): 94-103.
- Maffei, M, Halaas, J, Ravussin, E, Pratley, RE, Lee, GH, Zhang, Y, Fei, H, Kim, S, Lallone, R, Ranganathan, S (1995) Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nature Medicine*, 1(11): 1155-1161.
- Mailman, R, Murthy, V (2010) Third generation antipsychotic drugs: partial agonism or receptor functional selectivity? *Current Pharmaceutical Design*, 16(5): 488-501.
- Maric, T, Cantor, A, Cuccioletta, H, Tobin, S, Shalev, U (2009) Neuropeptide Y augments cocaine self-administration and cocaine-induced hyperlocomotion in rats. *Peptides*, 30(4): 721-726.
- Marini, P, Moriello, AS, Cristino, L, Palmery, M, De Petrocellis, L, Di Marzo, V (2009) Cannabinoid CB1 receptor elevation of intracellular calcium in neuroblastoma SH-SY5Y cells: Interactions with muscarinic and [delta]-opioid receptors. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1793(7): 1289-1303.

- Martin, DL, Rimvall, K (1993) Regulation of γ -Aminobutyric Acid Synthesis in the Brain. *Journal of Neurochemistry*, 60(2): 395-407.
- Martin, NM, Small, CJ, Sajedi, A, Liao, XH, Weiss, RE, Gardiner, JV, Ghatei, MA, Bloom, SR (2004) Abnormalities of the hypothalamo-pituitary-thyroid axis in the pro-opiomelanocortin deficient mouse. *Regulatory Peptides*, 122(3): 169-172.
- Matsui-Sakata, A, Ohtani, H, Sawada, Y (2005) Receptor occupancy-based analysis of the contributions of various receptors to antipsychotics-induced weight gain and diabetes mellitus. *Drug metabolism and pharmacokinetics*, 20(5): 368-378.
- Meeker, RB, Myers, RD (1980) GABA and glutamate: Possible metabolic intermediaries involved in the hypothalamic regulation of food intake. *Brain Research Bulletin*, 5(Suppl. 2): 253-259.
- Meister, B (2007) Neurotransmitters in key neurons of the hypothalamus that regulate feeding behavior and body weight. *Physiology and Behavior*, 92(1-2): 263-271.
- Meister, B, Gömüç, B, Suarez, E, Ishii, Y, Dürr, K, Gillberg, L (2006) Hypothalamic proopiomelanocortin (POMC) neurons have a cholinergic phenotype. *European Journal of Neuroscience*, 24(10): 2731-2740.
- Meltzer, HY (1992) Treatment of the Neuroleptic-Nonresponsive Schizophrenic Patient. *Schizophrenia Bulletin*, 18(3): 515-542.
- Menzies, JRW, Ludwig, M, Leng, G (2010) Direct and Indirect Effects of Cannabinoids on in vitro GABA Release in the Rat Arcuate Nucleus. *Journal of Neuroendocrinology*, 22(6): 585-592.
- Michel, AD, Stefanich, E, Whiting, RL (1989) Direct labeling of rat M3-muscarinic receptors by [3H]4DAMP. *European Journal of Pharmacology*, 166(3): 459-466.
- Millar, J (1963) A Trial of Fluphenazine in Schizophrenia. *The British Journal of Psychiatry*, 109(460): 428-432.
- Miller, CC, Murray, TF, Freeman, KG, Edwards, GL (2004) Cannabinoid agonist, CP 55,940, facilitates intake of palatable foods when injected into the hindbrain. *Physiology and Behavior*, 80(5): 611-616.
- Minet-Ringuet, J, Even, P, Lacroix, M, Tomé, D, de Beaurepaire, R (2006a) A model for antipsychotic-induced obesity in the male rat. *Psychopharmacology*, 187(4): 447-454.
- Minet-Ringuet, J, Even, PC, Goubert, M, Tomé, D, de Beaurepaire, R (2006b) Long term treatment with olanzapine mixed with the food in male rats induces body fat deposition with no increase in body weight and no thermogenic alteration. *Appetite*, 46(3): 254-262.
- Minet-Ringuet, J, Even, PC, Guesdon, B, Tomé, D, de Beaurepaire, R (2005) Effects of chronic neuroleptic treatments on nutrient selection, body weight, and body composition in the male rat under dietary self-selection. *Behavioural Brain Research*, 163(2): 204-211.
- Minor, RK, Chang, JW, de Cabo, R (2009) Hungry for life: How the arcuate nucleus and neuropeptide Y may play a critical role in mediating the benefits of calorie restriction. *Molecular and Cellular Endocrinology*, 299(1): 79-88.
- Morris, MJ, Chen, H, Watts, R, Shulkes, A, Cameron-Smith, D (2007) Brain neuropeptide Y and CCK and peripheral adipokine receptors: temporal response in obesity induced by palatable diet. *International Journal of Obesity*, 32(2): 249-258.
- Morrison, CD (2008) Leptin resistance and the response to positive energy balance. *Physiology and Behavior*, 94(5): 660-663.
- Munro, S, Thomas, K, Abu-Shaar, M (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, 365: 61-65.
- Murashita, M, Kusumi, I, Hosoda, H, Kangawa, K, Koyama, T (2007) Acute administration of clozapine concurrently increases blood glucose and circulating plasma ghrelin levels in rats. *Psychoneuroendocrinology*, 32(7): 777-784.

- Murashita, M, Kusumi, I, Inoue, T, Takahashi, Y, Hosoda, H, Kangawa, K, Koyama, T (2005) Olanzapine increases plasma ghrelin level in patients with schizophrenia. *Psychoneuroendocrinology*, 30: 106-110.
- Naber, D, Lambert, M (2004) Aripiprazole: a new atypical antipsychotic with a different pharmacological mechanism. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 28(8): 1213-1219.
- Narushima, M, Hashimoto, K, Kano, M (2006) Endocannabinoid-mediated short-term suppression of excitatory synaptic transmission to medium spiny neurons in the striatum. *Neuroscience Research*, 54(3): 159-164.
- Nasrallah, H (2003) A review of the effect of atypical antipsychotics on weight. *Psychoneuroendocrinology*, 28(Suppl. 1): 83-96.
- Nasrallah, H (2008) Atypical antipsychotic-induced metabolic side effects: insights from receptor-binding profiles. *Molecular Psychiatry*, 13(1): 27-35.
- Nemeroff, C (1997) Dosing the antipsychotic medication olanzapine. *Journal of Clinical Psychiatry*, 58(Suppl. 10): 45-49.
- Newcomer, J (2005) Second-Generation Atypical Antipsychotics and Metabolic Effects. A Comprehensive Literature Review. *CNS Drugs*, 19: 1-93.
- Nguyen, Q, Wagner, E (2007) Estrogen differentially modulates the cannabinoid- induced presynaptic inhibition of amino acid neurotransmission in proopiomelanocortin neurons of the arcuate nucleus. *Neuroendocrinology*, 84(2): 123.
- Nogueiras, R, Diaz-Arteaga, A, Lockie, SH, Velásquez, DA, Tschöp, J, López, M, Cadwell, CC, Diéguez, C, Tschöp, MH (2009) The endocannabinoid system: Role in glucose and energy metabolism. *Pharmacological Research*, 60(2): 93-98.
- O'Hare, JD, Zielinski, E, Cheng, B, Scherer, T, Buettner, C (2011) Central Endocannabinoid Signaling Regulates Hepatic Glucose Production and Systemic Lipolysis. *Diabetes*, 60(4): 1055-1062.
- O'Neill, MF (2005) Unusual suspect for antipsychotic-induced diabetes. *Drug Discovery Today*, 10(20): 1338-1338.
- Obici, S, Feng, Z, Arduini, A, Conti, R, Rossetti, L (2003) Inhibition of hypothalamic carnitine palmitoyltransferase-1 decreases food intake and glucose production. *Nature Medicine*, 9: 756-761.
- Obici, S, Feng, Z, Karkanias, G, Baskin, D, Rossetti, L (2002) Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nature Neuroscience*, 5(6): 566-572.
- Obuchowicz, E (1996) Long-term treatment with chlorpromazine and haloperidol but not with sulpiride and clozapine markedly elevates neuropeptide Y-like immunoreactivity in the rat hypothalamus. *Neuropeptides*, 30(5): 471-478.
- Obuchowicz, E, Krysiak, R, Herman, ZS (2004) Does neuropeptide Y (NPY) mediate the effects of psychotropic drugs? *Neuroscience and Biobehavioral Reviews*, 28(6): 595-610.
- Obuchowicz, E, Krysiak, R, Wieronska, JM, Smialowska, M, Herman, ZS (2005) Alterations in striatal neuropeptide Y system activity of rats with haloperidol-induced behavioral supersensitivity. *Neuropeptides*, 39(5): 515-523.
- Obuchowicz, E, Turchan, J (1998) Influence of typical and atypical antipsychotics on neuropeptide Y-like immunoreactivity and NPY mRNA expression in rat striatum. *Neuropeptides*, 32(5): 473-480.
- Obuchowicz, E, Turchan, J (1999) Clozapine decreases neuropeptide Y-like immunoreactivity and neuropeptide Y mRNA levels in rat nucleus accumbens. *European Neuropsychopharmacology*, 9(4): 329-335.

- Ochi, Y, Horie, S, Maruyama, T, Watanabe, K, Yano, S (2005) Necessity of intracellular cyclic AMP in inducing gastric acid secretion via muscarinic M3 and cholecystokinin2 receptors on parietal cells in isolated mouse stomach. *Life Sciences*, 77(16): 2040-2050.
- Ohno-Shosaku, T, Tsubokawa, H, Mizushima, I, Yoneda, N, Zimmer, A, Kano, M (2002) Presynaptic Cannabinoid Sensitivity Is a Major Determinant of Depolarization-Induced Retrograde Suppression at Hippocampal Synapses. *The Journal of Neuroscience*, 22(10): 3864-3872.
- Okumura, T, Namiki, M (1990) Vagal motor neurons innervating the stomach are site-specifically organized in the dorsal motor nucleus of the vagus nerve in rats. *Journal of the Autonomic Nervous System*, 29(2): 157-162.
- Oriot, P, Feys, J, Mertens de Wilmars, S, Misson, S, Ayache, A, Fagnart, O, Gruson, D, Luts, A, Jamart, J, Hermans, M-P, Buysschaert, M (2008) Insulin sensitivity, adjusted beta-cell function and adiponectinaemia among lean drug-naïve schizophrenic patients treated with atypical antipsychotic drugs: a nine-month prospective study. *Diabetes and Metabolism*, 34(5): 490-496.
- Orr, J, Davy, B (2005) Dietary Influences on Peripheral Hormones Regulating Energy Intake: Potential Applications for Weight Management. *Journal of the American Dietetic Association*, 105(7): 1115-1124.
- Ota, M, Mori, K, Nakashima, A, Kaneko, Y, Fujiwara, K, Itoh, M, Nagasaka, A, Ota, A (2002) Peripheral injection of risperidone, an atypical antipsychotic, alters the bodyweight gain of rats. *Clinical and Experimental Pharmacology and Physiology*, 29(11): 980-989.
- Pardini, AW, Nguyen, HT, Figlewicz, DP, Baskin, DG, Williams, DL, Kim, F, Schwartz, MW (2006) Distribution of insulin receptor substrate-2 in brain areas involved in energy homeostasis. *Brain Research*, 1112(1): 169-178.
- Park, S, Hong, S, Ahn, I, Kim, D, Kim, S (2010) Estrogen Replacement Reverses Olanzapine-Induced Weight Gain and Hepatic Insulin Resistance in Ovariectomized Diabetic Rats. *Neuropsychobiology*, 61(3): 148.
- Patil, B, Kulkarni, N, Unger, B (2006) Elevation of systolic blood pressure in an animal model of olanzapine induced weight gain. *European Journal of Pharmacology*, 551: 112-115.
- Pavlov, I (1941) *Lectures on conditioned reflexes*. London: Lawrence and Wishart.
- Paxinos, G, Watson, C (2007) *The Rat Brain in Stereotaxic Coordinates*. CA, USA: Elsevier Academic Press Inc.
- Pedrazzini, T, Seydoux, J, Kunstner, P, Aubert, J-F, Grouzmann, E, Beermann, F, Brunner, H-R (1998) Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor. *Nature Medicine*, 4(6): 722-726.
- Pelletier, G, Simard, J (1991) Dopaminergic regulation of pre-proNPY mRNA levels in the rat arcuate nucleus. *Neuroscience Letters*, 127(1): 96-98.
- Pelleymounter, MA, Cullen, MJ, Baker, MB, Hecht, R, Winters, D, Boone, T, Collins, F (1995) Effects of the obese gene product on body weight regulation in ob/ob mice. *Science*, 269(5223): 540-543.
- Perez-Iglesias, R, Vazquez-Barquero, JL, Amado, JA, Berja, A, Garcia-Unzueta, MT, Pelayo-Teran, JM, Carrasco-Marin, E, Mata, I, Crespo-Facorro, B (2008) Effect of antipsychotics on peptides involved in energy balance in drug-naïve psychotic patients after 1 year of treatment. *Journal of Clinical Psychopharmacology*, 28(3): 289-295.
- Pertwee, RG (1997) Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacology & Therapeutics*, 74(2): 129-180.
- Peruzzo, B, Pastor, F, Blazquez, J, Schobitz, K, Pelaez, B, P, A, Rodriguez, E (2000) A second look at the barriers of the medial basal hypothalamus. *Experimental Brain Research*, 132: 10-12.

- Pickar, D, Vinik, J, Bartko, JJ (2008) Pharmacotherapy of Schizophrenic Patients: Preponderance of Off-Label Drug Use. *PLoS ONE*, 3(9): e3150.
- Plum, L, Belgardt, BF, Brüning, JC (2006) Central insulin action in energy and glucose homeostasis. *The Journal of Clinical Investigation*, 116(7): 1761-1766.
- Polonsky, K, Given, B, Hirsch, L, Shapiro, E, Tillil, H, Beebe, C, JA, G, Frank, B, Karrison, T, Van Cauter, E (1988) Quantitative study of insulin secretion and clearance in normal and obese subjects. *Journal of Clinical Investigation*, 81(2): 435-441.
- Popovic, V, Doknic, M, Maric, N, Pekic, S, Damjanovic, A, Miljic, D, Popovic, S, Miljic, N, Djurovic, M, Jasovic-Gasic, M, Dieguez, C, Casanueva, FF (2007) Changes in Neuroendocrine and Metabolic Hormones Induced by Atypical Antipsychotics in Normal-Weight Patients with Schizophrenia. *Neuroendocrinology*, 85(4): 249-256.
- Pouzet, B, Mow, T, Kreilgård, M, Velschow, S (2003) Chronic treatment with antipsychotics in rats as a model for antipsychotic-induced weight gain in human. *Pharmacology, Biochemistry and Behavior*, 75(1): 133-140.
- Poyraz, BÇ, Aksoy, C, Balcloglu, I (2008) Increased incidence of autoimmune thyroiditis in patients with antipsychotic-induced hyperprolactinemia. *European Neuropsychopharmacology*, 18(9): 667-672.
- Procysbyn, RM, Chau, A, Tse, G (2004) Clozapine's effects on body weight and resting metabolic rate: a case series. *Schizophrenia Research*, 66(2-3): 159-162.
- Qian, S, Chen, H, Weingarh, D, Trumbauer, ME, Novi, DE, Guan, X, Yu, H, Shen, Z, Feng, Y, Frazier, E, Chen, A, Camacho, RE, Shearman, LP, Gopal-Truter, S, MacNeil, DJ, Van der Ploeg, LHT, Marsh, DJ (2002) Neither Agouti-Related Protein nor Neuropeptide Y Is Critically Required for the Regulation of Energy Homeostasis in Mice. *Molecular Cell Biology*, 22(14): 5027-5035.
- Qiu, J, Xue, C, Bosch, MA, Murphy, JG, Fan, W, RÅ_nnekleiv, OK, Kelly, MJ (2007) Serotonin 5-Hydroxytryptamine_{2C} Receptor Signaling in Hypothalamic Proopiomelanocortin Neurons: Role in Energy Homeostasis in Females. *Molecular Pharmacology*, 72(4): 885-896.
- Rader, DJ (2007) Effect of Insulin Resistance, Dyslipidemia, and Intra-abdominal Adiposity on the Development of Cardiovascular Disease and Diabetes Mellitus. *The American Journal of Medicine*, 120(3, Suppl. 1): S12-S18.
- Raedler, TJ, Bymaster, FP, Tandon, R, Copolov, D, Dean, B (2007) Towards a muscarinic hypothesis of schizophrenia. *Molecular Psychiatry*, 12(3): 232-246.
- Ramankutty, G (2002) Olanzapine-induced destabilization of diabetes in the absence of weight gain. *Acta Psychiatrica Scandinavica*, 105(3): 235-236.
- Raskind, MA, Burke, BL, Crites, NJ, Tapp, AM, Rasmussen, DD (2006) Olanzapine-Induced Weight Gain and Increased Visceral Adiposity is Blocked by Melatonin Replacement Therapy in Rats. *Neuropsychopharmacology*, 32(2): 284-288.
- Reagan-Shaw, S, Nihal, M, Ahmad, N (2007) Dose translation from animal to human studies revisited. *The FASEB Journal*, 22: 659-661.
- Remington, G, Mann, S, McCormick, P, Nobrega, JN, Hahn, M, Natesan, S (2011) Modeling chronic olanzapine exposure using osmotic minipumps: Pharmacological limitations. *Pharmacology Biochemistry and Behavior*, 100(1): 86-89.
- Renuka, TR, Ani, DV, Paulose, CS (2004) Alterations in the muscarinic M1 and M3 receptor gene expression in the brain stem during pancreatic regeneration and insulin secretion in weanling rats. *Life Sciences*, 75(19): 2269-2280.
- Reynolds, GP (1998) Receptor mechanisms of antipsychotic drug atypicality. *European Psychiatry*, 13(Suppl. 1): 5S-8S.
- Reynolds, GP, Kirk, SL (2010) Metabolic side effects of antipsychotic drug treatment - pharmacological mechanisms. *Pharmacology & Therapeutics*, 125(1): 169-179.

- Richelson, E, Souder, T (2000) Binding of antipsychotic drugs to human brain receptors: Focus on newer generation compounds. *Life Sciences*, 68(1): 29-39.
- Rogers, RC, McTigue, DM, Hermann, GE (1996) Vagal control of digestion: Modulation by central neural and peripheral endocrine factors. *Neuroscience and Biobehavioral Reviews*, 20(1): 57-66.
- Rohner-Jeanrenaud, F, Jeanrenaud, B (1980) Consequences of ventromedial hypothalamic lesions upon insulin and glucagon secretion by subsequently isolated perfused pancreases in the rat. *Journal of Clinical Investigation*, 65: 902-910.
- Roseberry, AG, Liu, H, Jackson, AC, Cai, X, Friedman, JM (2004) Neuropeptide Y-Mediated Inhibition of Proopiomelanocortin Neurons in the Arcuate Nucleus Shows Enhanced Desensitization in ob/ob Mice. *Neuron*, 41(5): 711-722.
- Roth, B, Driscoll, J (2011) NIMH Psychoactive Drug Screening Program.
- Roth, B, Kroeze, W, Patel, S, Lopez, E (2000) The Multiplicity of Serotonin Receptors: Uselessly diverse molecules or an embarrassment of riches? *The Neuroscientist*, 6: 252-262.
- Ruiz de Azua, I, Gautam, D, Guettier, J-M, Wess, J (2011) Novel insights into the function of [beta]-cell M3 muscarinic acetylcholine receptors: therapeutic implications. *Trends in Endocrinology & Metabolism*, 22(2): 74-80.
- Ryan, MCM, Flanagan, S, Kinsella, U, Keeling, F, Thakore, JH (2004) The effects of atypical antipsychotics on visceral fat distribution in first episode, drug-naïve patients with schizophrenia. *Life Sciences*, 74(16): 1999-2008.
- Sacher, J, Mossaheb, N, Spindelegger, C, Klein, N, Geiss-Granadia, T, Sauermann, R, Lackner, E, Joukhadar, C, Muller, M, Kasper, S (2008) Effects of olanzapine and ziprasidone on glucose tolerance in healthy volunteers. *Neuropsychopharmacology*, 33(7): 1633-1641.
- Savoy, YE, Ashton, MA, Miller, MW, Nedza, FM, Spracklin, DK, Hawthorn, MH, Rollemma, H, Matos, FF, Hajos-Korcsok, E (2008) Differential Effects of Various Typical and Atypical Antipsychotics on Plasma Glucose and Insulin Levels in the Mouse: Evidence for the Involvement of Sympathetic Regulation. *Schizophrenia Bulletin*, 36(2): 410-418.
- Scarr, E, Cowie, TF, Kanellakis, S, Sundram, S, Pantelis, C, Dean, B (2009) Decreased cortical muscarinic receptors define a subgroup of subjects with schizophrenia. *Molecular Psychiatry*, 14(11): 1017-1023.
- Scarr, E, Dean, B (2009) Role of the cholinergic system in the pathology and treatment of schizophrenia. *Expert Review of Neurotherapeutics*, 9(1): 73-86.
- Scarr, E, Keriakous, D, Crossland, N, Dean, B (2006) No change in cortical muscarinic M2, M3 receptors or [35S]GTP[gamma]S binding in schizophrenia. *Life Sciences*, 78(11): 1231-1237.
- Schleimer, SB, Johnston, GAR, Henderson, JM (2005) Novel oral drug administration in an animal model of neuroleptic therapy. *Journal of Neuroscience Methods*, 146(2): 159-164.
- Schwartz, MW (2001) Progress in the search for neuronal mechanisms coupling type 2 diabetes to obesity. *Journal of Clinical Investigation*, 108: 963-964.
- Schwartz, MW, Porte, D, Jr. (2005) Diabetes, Obesity, and the Brain. *Science*, 307(5708): 375-379.
- Schwartz, MW, Sipols, AJ, Marks, JL, Sanacora, G, White, JD, Scheurink, A, Kahn, SE, Baskin, DG, Woods, SC, Figlewicz, DP (1992) Inhibition of hypothalamic neuropeptide Y gene expression by insulin. *Endocrinology*, 130(6): 3608-3616.
- Schwartz, MW, Woods, SC, Porte, D, Seeley, RJ, Baskin, DG (2000) Central nervous system control of food intake. *Nature*, 404(6778): 661-671.
- Secher, A, Husum, H, Holst, B, Egerod, KL, Møllerup, E (2010) Risperidone Treatment Increases CB1 Receptor Binding in Rat Brain. *Neuroendocrinology*, 91(2): 155.
- Seeman, MV (2010) Schizophrenia: Women Bear a Disproportionate Toll of Antipsychotic Side Effects. *Journal of the American Psychiatric Nurses Association*, 16(1): 21-29.

- Sentissi, O, Epelbaum, J, Olie, J-P, Poirier, M-F (2008) Leptin and ghrelin levels in patients with schizophrenia during different antipsychotics treatment: a review. *Schizophrenia Bulletin*, 34(6): 1189-1199.
- Sernyak, MJ, Leslie, DL, Alarcon, RD, Losonczy, MF, Rosenheck, R (2002) Association of Diabetes Mellitus With Use of Atypical Neuroleptics in the Treatment of Schizophrenia. *American Journal of Psychiatry*, 159(4): 561-566.
- Sharkey, KA, Pittman, QJ (2005) Central and Peripheral Signaling Mechanisms Involved in Endocannabinoid Regulation of Feeding: A Perspective on the Munchies. *Science's STKE*, (277): pe15.
- Sharpe, J-K, Byrne, NM, Stedman, TJ, Hills, AP (2005) Resting energy expenditure is lower than predicted in people taking atypical antipsychotic medication. *Journal of the American Dietetic Association*, 105(4): 612-615.
- Shen, K-Z, Johnson, SW (2000) Presynaptic dopamine D2 and muscarine M3 receptors inhibit excitatory and inhibitory transmission to rat subthalamic neurones in vitro. *The Journal of Physiology*, 525(2): 331-341.
- Shen, WW (1999) A history of antipsychotic drug development. *Comprehensive Psychiatry*, 40(6): 407-414.
- Shertzer, HG, Kendig, EL, Nasrallah, HA, Johansson, E, Genter, MB (2010) Protection from olanzapine-induced metabolic toxicity in mice by acetaminophen and tetrahydroindenoindole. *International Journal of Obesity*, 34(6): 970-979.
- Shiia, T, Nakazato, M, Mizuta, M, Date, Y, Mondal, MS, Tanaka, M, Nozoe, S-I, Hosoda, H, Kangawa, K, Matsukura, S (2002) Plasma Ghrelin Levels in Lean and Obese Humans and the Effect of Glucose on Ghrelin Secretion. *Journal of Clinical Endocrinology and Metabolism*, 87(1): 240-244.
- Shin, AC, Zheng, H, Berthoud, H-R (2009) An expanded view of energy homeostasis: Neural integration of metabolic, cognitive, and emotional drives to eat. *Physiology and Behavior*, 97(5): 572-580.
- Shobo, M, Yamada, H, Mihara, T, Kondo, Y, Irie, M, Harada, K, Ni, K, Matsuoka, N, Kayama, Y (2010) Two models for weight gain and hyperphagia as side effects of atypical antipsychotics in male rats: Validation with olanzapine and ziprasidone. *Behavioural Brain Research*, 216(2): 561-568.
- Shrestha, YB, Wickwire, K, Giraudo, SQ (2009) Direct effects of nutrients, acetylcholine, CCK, and insulin on ghrelin release from the isolated stomachs of rats. *Peptides*, 30(6): 1187-1191.
- Silvestre, J, Prous, J (2005) Research on adverse drug events. I. Muscarinic M3 receptor binding affinity could predict the risk of antipsychotics to induce type 2 diabetes. *Methods and Findings in Experimental and Clinical Pharmacology*, 27(5): 289-304.
- Skouroliaou, M, Giannopoulou, I, Kostara, C, Hannon, JC (2009) Effects of nutritional intervention on body weight and body composition of obese psychiatric patients taking olanzapine. *Nutrition*, 25(7-8): 729-735.
- Smith, G, Chaussade, C, Vickers, M, Jensen, J, Shepherd, P (2008) Atypical antipsychotic drugs induce derangements in glucose homeostasis by acutely increasing glucagon secretion and hepatic glucose output in the rat. *Diabetologia*, 51(12): 2309-2317.
- Smith, GP, Jerome, C, Cushin, BJ, Eterno, R, Simansky, KJ (1981) Abdominal vagotomy blocks the satiety effect of cholecystikinin in the rat. *Science*, 213(4511): 1036-1037.
- Sperlágh, B, Windisch, K, Andó, RD, Sylvester Vizi, E (2009) Neurochemical evidence that stimulation of CB1 cannabinoid receptors on GABAergic nerve terminals activates the dopaminergic reward system by increasing dopamine release in the rat nucleus accumbens. *Neurochemistry International*, 54(7): 452-457.

- Stanley, BG, Kyrkouli, SE, Lampert, S, Leibowitz, SF (1986) Neuropeptide Y chronically injected into the hypothalamus: A powerful neurochemical inducer of hyperphagia and obesity. *Peptides*, 7(6): 1189-1192.
- Starrenburg, FCJ, Bogers, JPAM (2009) How can antipsychotics cause diabetes mellitus? Insights based on receptor-binding profiles, humoral factors and transporter proteins. *European Psychiatry*, 24(3): 164-170.
- Ste Marie, L, Luquet, S, Cole, T, Palmiter, R (2005) Modulation of neuropeptide Y expression in adult mice does not affect feeding. *Proceedings of the National Academy of Sciences*, 102(51): 18632-18637. Epub 12005 Dec 18638.
- Stefanidis, A, Verty, ANA, Allen, AM, Owens, NC, Cowley, MA, Oldfield, BJ (2009) The role of thermogenesis in antipsychotic drug-induced weight gain. *Obesity*, 17(1): 16-24.
- Stellar, E (1954) The physiology of motivation. *Psychology Reviews*, 61(5).
- Stice, E, Spoor, S, Ng, J, Zald, DH (2009) Relation of obesity to consummatory and anticipatory food reward. *Physiology and Behavior*, 97(5): 551-560.
- Stornetta, RL, Guyenet, PG (1999) Distribution of glutamic acid decarboxylase mRNA-containing neurons in rat medulla projecting to thoracic spinal cord in relation to monoaminergic brainstem neurons. *The Journal of Comparative Neurology*, 407(3): 367-380.
- Suzuki, T, Uchida, H, Watanabe, K, Nakajima, S, Nomura, K, Takeuchi, H, Tanabe, A, Yagi, G, Kashima, H (2008) Effectiveness of antipsychotic polypharmacy for patients with treatment refractory schizophrenia: an open-label trial of olanzapine plus risperidone for those who failed to respond to a sequential treatment with olanzapine, quetiapine and risperidone. *Human Psychopharmacology: Clinical and Experimental*, 23(6): 455-463.
- Tanaka, K, Morinobu, S, Ichimura, M, Asakawa, A, Inui, A, Hosoda, H, Kangawa, K, Yamawaki, S (2008) Decreased levels of ghrelin, cortisol, and fasting blood sugar, but not n-octanoylated ghrelin, in Japanese schizophrenic inpatients treated with olanzapine. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 32(6): 1527-1532.
- Tandon, R, Nasrallah, HA, Keshavan, MS (2009) Schizophrenia, "just the facts" 4. Clinical features and conceptualization. *Schizophrenia Research*, 110(1-3): 1-23.
- Tandon, R, Nasrallah, HA, Keshavan, MS (2010) Schizophrenia, "Just the Facts" 5. Treatment and prevention Past, present, and future. *Schizophrenia Research*, 122(1-3): 1-23.
- Tauscher, J, Jones, C, Remington, G, Zipursky, R, Kapur, S (2002) Significant dissociation of brain and plasma kinetics with antipsychotics. *Molecular Psychiatry*, 7: 317-321.
- Tebbe, JJ, Dietze, T, Grote, C, Mönnikes, H (2001) Excitatory stimulation of neurons in the arcuate nucleus inhibits gastric acid secretion via vagal pathways in anesthetized rats. *Brain Research*, 913(1): 10-17.
- Terry Jr, AV, Gearhart, DA, Mahadik, SP, Warsi, S, Waller, JL (2006) Chronic treatment with first or second generation antipsychotics in rodents: Effects on high affinity nicotinic and muscarinic acetylcholine receptors in the brain. *Neuroscience*, 140(4): 1277-1287.
- Thorens, B (2010) Central control of glucose homeostasis: the brain - endocrine pancreas axis. *Diabetes and Metabolism*, 36(Suppl. 3): S45-S49.
- Thornton-Jones, Z, Neill, JC, Reynolds, GP (2002) The atypical antipsychotic olanzapine enhances ingestive behaviour in the rat: a preliminary study. *Journal of Psychopharmacology*, 16(1): 35-37.
- Tiwari, AK, Rodgers, JB, Sicard, M, Zai, CC, Likhodi, O, Freeman, N, Meltzer, HY, Lieberman, JA, Kennedy, JL, Müller, DJ (2010a) Association study of polymorphisms in cholecystikinin gene and its receptors with antipsychotic induced weight gain in schizophrenia patients. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 34(8): 1484-1490.
- Tiwari, AK, Zai, CC, Likhodi, O, Lisker, A, Singh, D, Souza, RP, Batra, P, Zaidi, SHE, Chen, S, Liu, F, Puls, I, Meltzer, HY, Lieberman, JA, Kennedy, JL, Muller, DJ (2010b) A Common

- Polymorphism in the Cannabinoid Receptor 1 (CNR1) Gene is Associated with Antipsychotic-Induced Weight Gain in Schizophrenia. *Neuropsychopharmacology*, 35(6): 1315-1324.
- Tong, Q, Ye, C-P, Jones, JE, Elmquist, JK, Lowell, BB (2008) Synaptic release of GABA by AgRP neurons is required for normal regulation of energy balance. *Nature Neuroscience*, 11(9): 998-1000.
- Toshinai, K, Mondal, MS, Nakazato, M, Date, Y, Murakami, N, Kojima, M, Kangawa, K, Matsukura, S (2001) Upregulation of Ghrelin Expression in the Stomach upon Fasting, Insulin-Induced Hypoglycemia, and Leptin Administration. *Biochemical and Biophysical Research Communications*, 281(5): 1220-1225.
- Tost, H, Alam, T, Meyer-Lindenberg, A (2010) Dopamine and psychosis: Theory, pathomechanisms and intermediate phenotypes. *Neuroscience and Biobehavioral Reviews*, 34(5): 689-700.
- Travagli, RA, Hermann, GE, Browning, KN, Rogers, RC (2006) Brainstem circuits regulating gastric function. *Annual Review of Physiology*, 68: 279-305.
- Tschop, M, Weyer, C, Tataranni, PA, Devanarayan, V, Ravussin, E, Heiman, ML (2001) Circulating Ghrelin Levels Are Decreased in Human Obesity. *Diabetes*, 50(4): 707-709.
- Tulipano, G, Rizzetti, C, Bianchi, I, Fanzani, A, Spano, P, Cocchi, D (2007) Clozapine-Induced Alteration of Glucose Homeostasis in the Rat: The Contribution of Hypothalamic-Pituitary-Adrenal Axis Activation. *Neuroendocrinology*, 85(2): 61.
- Uchigashima, M, Narushima, M, Fukaya, M, Katona, I, Kano, M, Watanabe, M (2007) Subcellular Arrangement of Molecules for 2-Arachidonoyl-Glycerol-Mediated Retrograde Signaling and Its Physiological Contribution to Synaptic Modulation in the Striatum. *Journal of Neuroscience*, 27(14): 3663-3676.
- Unger, JW, Moss, AM, Livingston, JN (1991) Immunohistochemical localization of insulin receptors and phosphotyrosine in the brainstem of the adult rat. *Neuroscience*, 42(3): 853-861.
- Urban, JD, Vargas, GA, von Zastrow, M, Mailman, RB (2006) Aripiprazole has Functionally Selective Actions at Dopamine D2 Receptor-Mediated Signaling Pathways. *Neuropsychopharmacology*, 32(1): 67-77.
- Urbanski, MJ, Kovacs, FE, Szabo, B (2010) Endocannabinoid-mediated synaptically evoked suppression of GABAergic transmission in the cerebellar cortex. *Neuroscience*, 169(3): 1268-1278.
- Usall, J, Suarez, D, Haro, J (2007) Gender differences in response to antipsychotic treatment in outpatients with schizophrenia. *Psychiatry Research*, 153: 225-231.
- Valassi, E, Scacchi, M, Cavagnini, F (2008) Neuroendocrine control of food intake. *Nutrition, Metabolism and Cardiovascular Diseases*, 18(2): 158-168.
- van den Top, M, Lee, K, Whyment, AD, Blanks, AM, Spanswick, D (2004) Orexin-sensitive NPY/AgRP pacemaker neurons in the hypothalamic arcuate nucleus. *Nature Neuroscience*, 7: 493-494.
- van der Lely, AJ, Tschop, M, Heiman, ML, Ghigo, E (2004) Biological, Physiological, Pathophysiological, and Pharmacological Aspects of Ghrelin. *Endocrine Reviews*, 25(3): 426-457.
- van der Zwaal, EM, Lujendijk, MCM, Adan, RAH, la Fleur, SE (2008) Olanzapine-induced weight gain: Chronic infusion using osmotic minipumps does not result in stable plasma levels due to degradation of olanzapine in solution. *European Journal of Pharmacology*, 585(1): 130-136.
- van der Zwaal, EM, Lujendijk, MCM, Evers, SS, la Fleur, SE, Adan, RAH (2010) Olanzapine affects locomotor activity and meal size in male rats. *Pharmacology Biochemistry and Behavior*, 97(1): 130-137.

- Van Gaal, LF, Rissanen, AM, Scheen, AJ, Ziegler, O, Rossner, S (2005) Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *The Lancet*, 365(9468): 1389(1389).
- Van Sickle, MD, Duncan, M, Kingsley, PJ, Mouihate, A, Urbani, P, Mackie, K, Stella, N, Makriyannis, A, Piomelli, D, Davison, JS, Marnett, LJ, Di Marzo, V, Pittman, QJ, Patel, KD, Sharkey, KA (2005) Identification and Functional Characterization of Brainstem Cannabinoid CB2 Receptors. *Science*, 310(5746): 329-332.
- Van Sickle, MD, Oland, LD, Mackie, K, Davison, JS, Sharkey, KA (2003) {Delta}9-Tetrahydrocannabinol selectively acts on CB1 receptors in specific regions of dorsal vagal complex to inhibit emesis in ferrets. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, 285(3): G566-576.
- Verhagen, LAW, Luijendijk, MCM, Hillebrand, JJG, Adan, RAH Dopamine antagonism inhibits anorectic behavior in an animal model for anorexia nervosa. *European Neuropsychopharmacology*, In Press, Corrected Proof.
- Vidarsdottir, S, Roelfsema, F, Streefland, T, Holst, JJ, Rehfeld, JF, Pijl, H (2010) Short-term treatment with olanzapine does not modulate gut hormone secretion: olanzapine disintegrating versus standard tablets. *European Journal of Endocrinology*, 162(1): 75-83.
- Virgo, L, Humphries, C, Mortimer, A, Barnes, T, Hirsch, S, de Belleruche, J (1995) Cholecystokinin messenger RNA deficit in frontal and temporal cerebral cortex in schizophrenia. *Biological Psychiatry*, 37(10): 694-701.
- Virkkunen, M, Wahlbeck, K, Rissanen, A, Naukkarinen, H, Franssila-Kallunki, A (2002) Decrease of Energy Expenditure Causes Weight Increase in Olanzapine Treatment - A Case Study. *Pharmacopsychiatry*, 35(03): 124,126.
- Vitiello, B, Correll, C, van Zwieten-Boot, B, Zuddas, A, Parellada, M, Arango, C (2009) Antipsychotics in children and adolescents: Increasing use, evidence for efficacy and safety concerns. *European Neuropsychopharmacology*, 19(9): 629-635.
- Vong, L, Ye, C, Yang, Z, Choi, B, Chua, S, Lowell, B (2011) Leptin Action on GABAergic Neurons Prevents Obesity and Reduces Inhibitory Tone to POMC Neurons. *Neuron*, 71(1): 142-154.
- Voruganti, L, Cortese, L, Oyewumi, L, Cernovsky, Z, Zirul, S, Awad, A (2000) Comparative evaluation of conventional and novel antipsychotic drugs with reference to their subjective tolerability, side-effect profile and impact on quality of life. *Schizophrenia Research*, 43(2-3): 135-145.
- Wai, S, Kindler, P, Lam, E, Zhang, A, Yew, D (2004) Distribution of neuropeptide Y-immunoreactive neurons in the human brainstem, cerebellum, and cortex during development. *Cellular and Molecular Neurobiology*, 24(5): 667-684.
- Walaas, I, Fonnum, F (1978) The effect of parenteral glutamate treatment on the localization of neurotransmitters in the mediobasal hypothalamus. *Brain Research*, 153: 549-562.
- Wang, Q, Huang, X-F (2008) Effects of chronic treatment of olanzapine and haloperidol on peptide YY binding densities in the rat brain. *Experimental Neurology*, 209(1): 261-267.
- Weiden, PJ, Mackell, JA, McDonnell, DD (2004) Obesity as a risk factor for antipsychotic noncompliance. *Schizophrenia Research*, 66(1): 51-57.
- Weston-Green, K, Huang, X-F, Deng, C (2010) Sensitivity of the female rat to olanzapine-induced weight gain--Far from the clinic? [letter]. *Schizophrenia Research*, 116(2-3): 299-300.
- Weston-Green, K, Huang, X-F, Deng, C (2011) Olanzapine treatment and metabolic dysfunction: a dose response study in female Sprague Dawley rats. *Behavioural Brain Research*, 217(2): 337-346.

- Weston-Green, K, Huang, X-F, Han, M, Deng, C (2008) The effects of antipsychotics on the density of cannabinoid receptors in the dorsal vagal complex of rats: implications for olanzapine-induced weight gain. *International Journal of Neuropsychopharmacology*, 11(06): 827-835.
- White, J, Olchovsky D, Kershaw M, M, B (1990) Increased hypothalamic content of preproneuropeptide-Y messenger ribonucleic acid in streptozotocin-diabetic rats. *Endocrinology*, 126(2): 765-772.
- Wiley, JL, Burston, JJ, Leggett, DC, Alekseeva, OO, Razdan, RK, Mahadevan, A, Martin, BR (2005) CB1 cannabinoid receptor-mediated modulation of food intake in mice. *British Journal of Pharmacology*, 145(3): 293.
- Williams, CM, Kirkham, TC (1999) Anandamide induces overeating: mediation by central cannabinoid (CB1) receptors. *Psychopharmacologia*, 143(3): 315-317.
- Wilson, RI, Nicoll, RA (2002) Endocannabinoid signaling in the brain. (Review: Neuroscience). *Science*, 296(5568): 678(675).
- Wirshing, DA, Pierre, JM, Marder, SR, Saunders, CS, Wirshing, WC (2002) Sexual side effects of novel antipsychotic medications. *Schizophrenia Research*, 56(1-2): 25-30.
- Wolkin, A, Barouche, F, Wolf, A, Rotrosen, J, Fowler, J, Shiue, C-Y, Cooper, T, Brodie, J (1989) Dopamine blockade and clinical response: evidence for two biological subgroups of schizophrenia. *The American Journal of Psychiatry*, 146: 905-908.
- Woods, SC, Porte, D, Jr., Bobbioni, E, Ionescu, E, Sauter, JF, Rohner-Jeanrenaud, F, Jeanrenaud, B (1985) Insulin: its relationship to the central nervous system and to the control of food intake and body weight. *American Journal of Clinical Nutrition*, 42(5): 1063-1071.
- Wren, AM, Bloom, SR (2007) Gut Hormones and Appetite Control. *Gastroenterology*, 132(6): 2116-2130.
- Wren, AM, Seal, LJ, Cohen, MA, Brynes, AE, Frost, GS, Murphy, KG, Dhillon, WS, Ghatei, MA, Bloom, SR (2001) Ghrelin Enhances Appetite and Increases Food Intake in Humans. *Journal of Clinical Endocrinology and Metabolism*, 86(12): 5992-.
- Wren, AM, Small, CJ, Ward, HL, Murphy, KG, Dakin, CL, Taheri, S, Kennedy, AR, Roberts, GH, Morgan, DGA, Ghatei, MA, Bloom, SR (2000) The Novel Hypothalamic Peptide Ghrelin Stimulates Food Intake and Growth Hormone Secretion. *Endocrinology*, 141(11): 4325-4328.
- Wu, Q, Palmiter, RD (2011) GABAergic signaling by AgRP neurons prevents anorexia via a melanocortin-independent mechanism. *European Journal of Pharmacology*, 660(1): 21-27.
- Wu, R-R, Zhao, J-P, Zhai, J-G, Guo, X-F, Guo, W-B (2007) Sex difference in effects of typical and atypical antipsychotics on glucose-insulin homeostasis and lipid metabolism in first-episode schizophrenia. *Journal of Clinical Psychopharmacology*, 27(4): 374-379.
- Wu, R, Zhao, J, Guo, X, He, Y, Fang, M, Guo, W, Chen, J, Li, L (2008) Metformin addition attenuates olanzapine-induced weight gain in drug-naïve first-episode schizophrenia patients: a double-blind, placebo-controlled study. *American Journal of Psychiatry*, 165(3): 352-358.
- Wu, X, Gao, J, Yan, J, Owyang, C, Li, Y (2004) Hypothalamus–Brain Stem Circuitry Responsible for Vagal Efferent Signaling to the Pancreas Evoked By Hypoglycemia in Rat. *Journal of Neurophysiology*, 91(4): 1734-1747.
- Wuchert, F, Ott, D, Rafalzik, S, Roth, J, Gerstberger, R (2009) Tumor necrosis factor- α , interleukin-1 β and nitric oxide induce calcium transients in distinct populations of cells cultured from the rat area postrema. *Journal of Neuroimmunology*, 206(1-2): 44-51.
- Xu, B, Kalra, PS, Farmerie, WG, Kalra, SP (1999) Daily Changes in Hypothalamic Gene Expression of Neuropeptide Y, Galanin, Proopiomelanocortin, and Adipocyte Leptin

- Gene Expression and Secretion: Effects of Food Restriction. *Endocrinology*, 140(6): 2868-2875.
- Yahya, A, Xiao, C, Chance, WT, Sheriff, S (2006) Up-regulation of neuropeptide Y Y4 receptor mRNA expression in the brainstem of refed rats following 48 h of food deprivation: Effect of leptin. *Peptides*, 27(11): 2731-2737.
- Yamada, M, Miyakawa, T, Duttaroy, A, Yamanaka, A, Moriguchi, T, Makita, R, Ogawa, M, Chou, CJ, Xia, B, Crawley, JN, Felder, CC, Deng, C-X, Wess, J (2001) Mice lacking the M3 muscarinic acetylcholine receptor are hypophagic and lean. *Nature*, 410(6825): 207-212.
- Yang, L-H, Chen, T-M, Yu, S-T, Chen, Y-H (2007) Olanzapine induces SREBP-1-related adipogenesis in 3T3-L1 cells. *Pharmacological Research*, 56(3): 202-208.
- Yang, T-T, Chang, C-K, Tsao, C-W, Hsu, Y-M, Hsu, C-T, Cheng, J-T (2009) Activation of muscarinic M-3 receptor may decrease glucose uptake and lipolysis in adipose tissue of rats. *Neuroscience Letters*, 451(1): 57-59.
- Yasumi, M, Sato, K, Shimada, S, Nishimura, M, Tohyama, M (1997) Regional distribution of GABA transporter 1 (GAT1) mRNA in the rat brain: Comparison with glutamic acid decarboxylase67 (GAD67) mRNA localization. *Molecular Brain Research*, 44(2): 205-218.
- Yonkers, K, Kando, J, Cole, J, Blumenthal, S (1992) Gender differences in pharmacokinetics and pharmacodynamics of psychotropic medication. *American Journal of Psychiatry*, 149(5): 587-595.
- Yoshimatsu, H (2006) The neuronal histamine H1 and pro-opiomelanocortin-melanocortin 4 receptors: Independent regulation of food intake and energy expenditure. *Peptides*, 27(2): 326-332.
- Yuan, P-Q, Yang, H (2002) Neuronal activation of brain vagal-regulatory pathways and upper gut enteric plexuses by insulin hypoglycemia. *American Journal of Physiology - Endocrinology And Metabolism*, 283(3): E436-448.
- Zavitsanou, K, Nguyen, V, Han, M, Huang, X (2007) Effects of Typical and Atypical Antipsychotic Drugs on Rat Brain Muscarinic Receptors. *Neurochemical Research*, 32(3): 525-532.
- Zawalich, WS, Zawalich, KC, Tesz, GJ, Taketo, MM, Sterpka, J, Philbrick, W, Matsui, M (2004) Effects of muscarinic receptor type 3 knockout on mouse islet secretory responses. *Biochemical and Biophysical Research Communications*, 315(4): 872-876.
- Zhang, Y, Proenca, R, Maffei, M, Barone, M, Leopold, L, Friedman, JM (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372(6505): 425-432.
- Zhang, Y, Rodrigues, E, Gao, YX, King, M, Cheng, KY, Erdös, B, Tümer, N, Carter, C, Scarpace, PJ (2010) Pro-opiomelanocortin gene transfer to the nucleus of the solitary tract but not arcuate nucleus ameliorates chronic diet-induced obesity. *Neuroscience*, 169(4): 1662-1671.
- Zhang, Z-J, Yao, Z-J, Liu, WEN, Fang, QUN, Reynolds, GP (2004) Effects of antipsychotics on fat deposition and changes in leptin and insulin levels: Magnetic resonance imaging study of previously untreated people with schizophrenia. *British Journal of Psychiatry*, 184(1): 58-62.
- Zheng, H, Patterson, LM, Phifer, CB, Berthoud, H-R (2005) Brain stem melanocortinerigic modulation of meal size and identification of hypothalamic POMC projections. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 289(1): R247-258.
- Zipursky, RB, Gu, H, Green, AI, Perkins, DO, Tohen, MF, McEvoy, JP, Strakowski, SM, Sharma, T, Kahn, RS, Gur, RE, Tollefson, GD, Lieberman, JA (2005) Course and predictors of weight gain in people with first-episode psychosis treated with olanzapine or haloperidol. *British Journal of Psychiatry*, 187(6): 537-543.

- Zirkle, C (1973) To tranquilizers and antidepressants: from antimalarials and antihistamines. In: Clarke, F (Ed.), *How Modern Medicines are Discovered* (pp. 55-77) Mt. Kisco, NY: Futura.
- Zubieta, JK, Frey, KA (1993) Autoradiographic mapping of M3 muscarinic receptors in the rat brain. *Journal of Pharmacology & Experimental Therapeutics*, 264(1): 415-422.
- Zuddas, A, Zanni, R, Usala, T (2011) Second generation antipsychotics (SGAs) for non-psychotic disorders in children and adolescents: A review of the randomized controlled studies. *European Neuropsychopharmacology*, 21(8): 600-620.
- Zwirska-Korczala, K, Konturek, S, Sodowski, M, Wylezol, M, Kuka, D, Sowa, P, Adamczyk-Sowa, M, Kukla, M, Berdowska, A, Rehfeld, J, Bielanski, W, Brzozowski, T (2007) Basal and postprandial plasma levels of PYY, ghrelin, cholecystokinin, gastrin and insulin in women with moderate and morbid obesity and metabolic syndrome. *Journal of Physiology and Pharmacology*, 58(Suppl. 1): 13-35.

APPENDIX ONE

A1.1 SENSITIVITY OF THE FEMALE RAT TO OLANZAPINE-INDUCED WEIGHT GAIN – FAR FROM THE CLINIC?

Reprinted from Schizophrenia Research, 116(2-3), Weston-Green K, Huang XF and Deng C,
Sensitivity of the Female Rat to olanzapine-induced weight gain – Far from the clinic?, 299-300,
Copyright (2010), with permission from Elsevier.



Letter to the Editors

Sensitivity of the female rat to olanzapine-induced weight gain—Far from the clinic?

The recent paper by [Chintoh et al. \(2008\)](#) reporting olanzapine-induced dysfunction in glucose metabolism, enhanced visceral fat and reduced locomotor activity in female rats was highly interesting as it illustrated olanzapine's ability to replicate aspects of metabolic dysfunction in the rodent model in a similar manner to the human scenario. However, contrary to previous reports in the rat and the clinic, the authors reported no change in body weight or food intake following olanzapine treatment, questioning the validity of the rat model. For the past decade scientists have worked to establish a rodent model that mimics the side-effect of metabolic dysfunction induced by some atypical antipsychotic drugs in the clinic, and some important experimental considerations have surfaced as a result. In particular, the issue of animal gender identified in previous animal model studies of olanzapine-induced weight gain ([Minet-Ringuet et al., 2006](#); [Pouzet et al., 2003](#)) shadows the legitimacy of the rodent model in its ability to mimic the human scenario. Indeed, reports indicate that male rats are less sensitive to olanzapine-induced weight gain than females. For example, contrary to female rats, male Sprague Dawley, Wistar and Mol:Wistar Hannover rats treated with olanzapine at a dosage range of 1–20 mg/kg/day failed to exhibit increased food intake or weight gain ([Albaugh et al., 2006](#); [Pouzet et al., 2003](#)). However, [Minet-Ringuet et al. \(2006\)](#) found that olanzapine (1 mg/kg) treatment for 6 weeks increased adiposity and circulating leptin levels in male rats, indicating that olanzapine's enhancement of adiposity and leptin can be replicated in the rat model for both sexes.

Based on clinical data, we suggest that the rodent model of olanzapine-induced weight gain mimics aspects of the human situation as studies have revealed gender-related differences in the human response to olanzapine treatment. Evidence shows that females with psychotic disorder have a 3.6-fold increased risk of weight gain than males ([Hakko et al., 2006](#)) and previous studies have identified female gender as a risk factor and predictor for weight gain associated with olanzapine and other atypical antipsychotics ([Gebhardt et al., 2009](#)). In fact, [Wu et al. \(2007\)](#) reported that female first-episode schizophrenia patients had a higher hip to waist ratio, increased insulin-resistance and higher plasma triglycerides than males following treatment with olanzapine and clozapine. [Kluge et al. \(2009\)](#) found that olanzapine significantly increased the BMI of

female patients after 1 week of treatment, however male patients took longer to reach significance, and females exhibited increased skin-fold thickness but not males. Females also showed a 2–4 times higher level of plasma leptin than males following olanzapine treatment, and this increase was observed earlier in females than in male patients ([Kluge et al., 2009](#)). Furthermore, female schizophrenia patients are more responsive to olanzapine treatment than male patients, regardless of illness chronicity ([Usall et al., 2007](#)). Female patients also exhibit higher plasma concentrations of the drug than males ([Kelly et al., 1999](#)), possibly due to their generally lower lean body mass and increased adipose tissue, allowing greater drug storage and leading to higher plasma levels over time ([Yonkers et al., 1992](#)). Gonadal steroids such as oestrogen, progesterone and testosterone can influence food intake and metabolism. [Fitzgerald et al. \(2003\)](#) identified significant positive correlations between changes in oestrogen levels and alterations in leptin and NPY levels as well as BMI and weight gain in female schizophrenia patients treated with olanzapine or risperidone. These results suggested that fluctuating gonadal steroid levels may play a role in the weight gain side-effect of atypical antipsychotic drugs and may explain the higher sensitivity of females to antipsychotic-induced weight gain, though the exact mechanism is unknown ([Fitzgerald et al., 2003](#)). Finally, based on waist circumference measurements, olanzapine-treated male patients are more responsive to nutritional intervention than females ([Skourliakou et al., 2009](#)).

Indeed, human studies have shown that atypical antipsychotic-induced weight gain does not occur in all patients, and findings from the large-scale Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study showed an increase of 7% weight gain from baseline in 30% of patients treated with olanzapine ([Allison et al., 2009](#)).

Taken together, the rodent model olanzapine-induced weight gain cannot completely replicate human weight gain side-effect, particularly in male rats. However, the sensitivity of female rodents to this side-effect over males appears to be a common observation in the clinic. Future studies on sex differences in the rodent model may improve our understanding of the mechanisms underlying gender response to antipsychotic effects.

Role of funding source

This study was supported by the University of Wollongong and the Schizophrenia Research Institute, Australia, utilising infrastructure funding from NSW Health; these sources had no further role in writing or the decision to submit the paper for publication.

Contributors

Weston-Green prepared the first draft of manuscript, with Deng and Huang providing important input in discussion and preparation of this manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

References

- Albaugh, V., Henry, C., Bello, N., Hajnal, A., Lynch, S., Halle, B., Lynch, C., 2006. Hormonal and metabolic effects of olanzapine and clozapine related to body weight in rodents. *Obesity* 14 (1), 36–50.
- Allison, D.B., Newcomer, J.W., Dunn, A.L., Blumenthal, J.A., Fabricatore, A.N., Daumit, G.L., Cope, M.B., Riley, W.T., Vreeland, B., Hibbeln, J.R., Alpert, J.E., 2009. Obesity among those with mental disorders: a national institute of mental health meeting report. *Am. J. Prev. Med.* 36 (4), 341–350.
- Chintoh, A.F., Mann, S.W., Lam, T.K.T., Giacca, A., Remington, G., 2008. Insulin resistance following continuous, chronic olanzapine treatment: an animal model. *Schizophr. Res.* 104 (1–3), 23–30.
- Fitzgerald, P., Scaffidi, A., Morris, M., de Castella, A., Kulkarni, J., 2003. The relationship of changes in leptin, neuropeptide Y and reproductive hormones to antipsychotic induced weight gain. *Hum. Psychopharmacol.* 18 (7), 551–557.
- Gebhardt, S., Haberhausen, M., Heinzl-Gutenbrunner, M., Gebhardt, N., Remschmidt, H., Krieg, J.-C., Hebebrand, J., Theisen, F.M., 2009. Antipsychotic-induced body weight gain: predictors and a systematic categorization of the long-term weight course. *J. Psychiatr. Res.* 43 (6), 620–626.
- Hakko, H., Komulainen, M.T., Koponen, H., Saari, K., Laitinen, J., Järvelin, M.-R., Lindeman, S., 2006. Are females at special risk of obesity if they become psychotic? The longitudinal Northern Finland 1966 Birth Cohort Study. *Schizophr. Res.* 84 (1), 15–19.
- Kelly, D.L., Conley, R.R., Tamminga, C.A., 1999. Differential olanzapine plasma concentrations by sex in a fixed-dose study. *Schizophr. Res.* 40 (2), 101–104.
- Kluge, M., Schuld, A., Schacht, A., Himmerich, H., Dalal, M.A., Wehmeier, P.M., Hinze-Selch, D., Kraus, T., Dittmann, R.W., Pollmächer, T., 2009. Effects of clozapine and olanzapine on cytokine systems are closely linked to weight gain and drug-induced fever. *Psychoneuroendocrinology* 34 (1), 118–128.
- Minet-Ringuet, J., Even, P.C., Goubern, M., Tomé, D., Beaurepaire, R.D., 2006. Long term treatment with olanzapine mixed with the food in male rats induces body fat deposition with no increase in body weight and no thermogenic alteration. *Appetite* 46 (3), 254–262.
- Pouzet, B., Mow, T., Kreilgård, M., Velschow, S., 2003. Chronic treatment with antipsychotics in rats as a model for antipsychotic-induced weight gain in human. *Pharmacol. Biochem. Behav.* 75 (1), 133–140.
- Skouroliaou, M., Giannopoulou, I., Kostara, C., Hannon, J.C., 2009. Effects of nutritional intervention on body weight and body composition of obese psychiatric patients taking olanzapine. *Nutrition* 25 (7–8), 729–735.
- Usall, J., Suarez, D., Haro, J., 2007. Gender differences in response to antipsychotic treatment in outpatients with schizophrenia. *Psychiatry Res.* 153, 225–231.
- Wu, R.-R., Zhao, J.-P., Zhai, J.-G., Guo, X.-F., Guo, W.-B., 2007. Sex difference in effects of typical and atypical antipsychotics on glucose-insulin homeostasis and lipid metabolism in first-episode schizophrenia. *J. Clin. Psychopharmacol.* 27 (4), 374–379.
- Yonkers, K., Kando, J., Cole, J., Blumenthal, S., 1992. Gender differences in pharmacokinetics and pharmacodynamics of psychotropic medication. *Am. J. Psychiatry* 149 (5), 587–595.

Katrina Weston-Green
Xu-Feng Huang
Chao Deng*

*Centre for Translational Neuroscience, School of Health Sciences,
University of Wollongong, Wollongong, 2522, NSW, Australia
Schizophrenia Research Institute, 384 Victoria Street,
Darlinghurst, 2010, NSW, Australia*

*Corresponding author. School of Health Sciences,
University of Wollongong, Wollongong, 2522, NSW, Australia.
Tel.: +61 2 4221 4934; fax: +61 2 4221 4096.
E-mail address: chao@uow.edu.au (C. Deng).

25 September 2009