

ACTIVATION AND MODULATION OF GABA(B) RECEPTOR SYSTEMS IN  
PHYSIOGENIC AND PATHOPHYSIOLOGICAL CONDITIONS

by

KIMBERLEY ANNE PITMAN

B.Sc. (Hons), University of Otago, 2009

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF  
THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES  
(Pharmacology and Therapeutics)

THE UNIVERSITY OF BRITISH COLUMBIA  
(Vancouver)

July 2015

©Kimberley Anne Pitman, 2015

## **Abstract**

$\gamma$ -aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system. This thesis explores the actions of known or proposed agonists for the GABA type B (GABA<sub>B</sub>) receptor in isolated cells and on nerve terminals releasing dopamine in rodents. The first chapter provides the background information for the research undertaken in this thesis. In the second chapter I used whole cell patch clamp electrophysiology to explore the action of the proposed atypical GABA<sub>B</sub> receptor agonist, R-isovaline, at heterologously or natively expressed GABA<sub>B</sub> receptors in isolated cells. I found that R-isovaline did not initiate GABA<sub>B</sub>-mediated K<sup>+</sup> currents and did not occlude or enhance the responses of other GABA<sub>B</sub> receptor agonists. Our data suggest that R-isovaline does not act as a direct agonist at GABA<sub>B</sub> receptors. In the third chapter I used fast-scan cyclic voltammetry in brain slices to explore the mechanisms behind GABA<sub>B</sub> modulation of dopamine release in the nucleus accumbens core of mice. The canonical GABA<sub>B</sub> receptor agonist, baclofen, concentration-dependently inhibited dopamine release by a direct action on dopamine neuron terminals. Baclofen-mediated inhibition of release was reduced when dopamine was released by stimulations that mimicked burst firing of dopamine neurons. Baclofen also decreased the probability of dopamine release. I also found that GABA<sub>B</sub> modulation of dopamine release was not altered by a sensitising cocaine treatment. In the fourth chapter I used fast scan cyclic voltammetry to assess whether diet induced obesity or a binge pattern of eating could alter GABA<sub>B</sub> or dopamine D2 receptor mediated regulation of dopamine release in the nucleus accumbens core in rats. The abilities of baclofen and the D2 agonist quinpirole to inhibit dopamine release were unchanged by long term access to a

palatable cafeteria diet. Chapter 5 discusses the main findings of these experiments and potential future experiments.

## **Preface**

My contributions to the second chapter include designing and performing all the experiments, analysing the data and writing the manuscript. A version of Chapter 2 has been published:

Pitman, K.A., Borgland, S.L., MacLeod, B., and Puil, E. (2015). Isovaline does not activate GABA(B) receptor-coupled potassium currents in GABA(B) expressing AtT-20 cells and cultured rat hippocampal neurons. *PLoS One* 10: e0118497. In addition to the work performed by KP; BM and EP helped conceive and design the experiments along with input and advice from SLB. SLB co-wrote the manuscript along with input and advice from EP and BM. Data are available online at: <http://figshare.com/s/37f4cd8a97b711e4ab1006ec4bbcf141>.

My contributions to the third chapter include designing and performing all the experiments, analysing the data and writing the manuscript. A version of Chapter 3 has been published:

Pitman, K.A., Puil, E., and Borgland, S.L. (2014). GABA B modulation of dopamine release in the nucleus accumbens core. *Eur. J. Neurosci.* 40: 3472–3480. In addition to the work performed by KP; SLB helped conceive and design the experiments and co-wrote the manuscript along with input and advice from EP.

My contributions to the fourth chapter include feeding and husbandry of the rats, designing and performing the voltammetry experiments, analysing all the data and writing the manuscript.

This research was approved by the University of British Columbia and University of Calgary animal care committees.

University of British Columbia animal care committee certificate number: A12-0089

Project Title: Mainly mice and neuropeptides

University of Calgary animal care committee certificate numbers: A13-0074, A13-0106

Project Titles: Experimental protocol for neuropeptide and hormonal modulation of neural mechanisms behind appetitive motivation; How are corticostriatal circuits modulated after compulsive eating in rats?

## Table of contents

|  |     |
|--|-----|
| Abstract.....  | ii  |
| Preface .....  | iv  |
| Table of contents .....  | vi  |
| List of tables .....   | x   |
| List of figures.....   | xi  |
| List of abbreviations.....   | xii |
| Acknowledgements.....  | xiv |
| Chapter 1 Introduction .....   | 1   |
| Overview and objectives.....   | 1   |
| Physiology of GABA <sub>B</sub> receptors.....   | 1   |
| Structure .....  | 2   |
| Function .....   | 6   |
| Regulation of GABA <sub>B</sub> responses.....   | 10  |
| Pharmacology of GABA <sub>B</sub> receptors .....  | 13  |
| Baclofen .....   | 13  |
| Allosteric modulation of the GABA <sub>B</sub> receptor .....  | 14  |
| Isovaline, an atypical GABA <sub>B</sub> agonist .....   | 15  |
| GABA <sub>B</sub> modulation of drug self-administration and rewarding effects of drugs of abuse ..... | 17  |
| GABA <sub>B</sub> regulation of food intake.....   | 20  |
| The mesolimbic dopamine system .....   | 22  |
| Role of NAc dopamine in behaviours .....   | 22  |
| Dopamine receptors .....   | 24  |
| Regulation of extracellular dopamine concentration.....  | 25  |
| Dopamine neuron firing.....  | 25  |
| Dopamine clearance .....   | 25  |
| Terminal modulation of dopamine release .....  | 26  |
| GABA <sub>B</sub> modulation of dopamine release.....  | 30  |
| Cocaine induced alterations in GABA <sub>B</sub> receptor expression and signalling .....              | 32  |

|  |    |
|--|----|
| Diet induced changes in the mesolimbic dopamine system .....   | 34 |
| Chapter 2 Isovaline does not activate GABA <sub>B</sub> receptor-coupled K <sup>+</sup> currents in either GABA <sub>B</sub> expressing AtT-20 cells or in cultured rat hippocampal neurons..... | 37 |
| Introduction .....   | 37 |
| Materials and methods.....   | 38 |
| AtT-20 cell culturing, transfection and electrophysiology.....   | 38 |
| Hippocampal cell culturing and electrophysiology.....  | 40 |
| Drugs .....  | 42 |
| Statistical analysis .....   | 42 |
| Results.....   | 43 |
| GABA <sub>B</sub> receptors express and are functional in transfected AtT-20 cells .....   | 43 |
| R-isovaline did not activate GABA <sub>B</sub> receptor coupled K <sup>+</sup> currents .....  | 46 |
| R-isovaline did not alter responses to other GABA <sub>B</sub> receptor agonists.....  | 48 |
| R-isovaline did not alter GABA <sub>B</sub> mediated K <sup>+</sup> currents in cultured hippocampal neurons .....   | 50 |
| Discussion.....  | 53 |
| R-isovaline did not mimic the actions of the other GABA <sub>B</sub> agonists .....  | 53 |
| Potential reasons for the lack of action of R-isovaline in our assay system.....   | 54 |
| Summary and future directions .....  | 56 |
| Chapter 3 GABA <sub>B</sub> modulation of dopamine release in the NAc core .....   | 57 |
| Introduction .....   | 57 |
| Materials and methods.....   | 58 |
| Subjects.....  | 58 |
| Slice preparation .....  | 59 |
| Cocaine administration.....  | 59 |
| Fast-scan cyclic voltammetry.....  | 60 |
| Drugs .....  | 61 |
| Statistical analysis .....   | 62 |
| Results.....   | 63 |
| Activation of GABA <sub>B</sub> receptors inhibits dopamine release in the NAc core .....  | 63 |

|  |    |
|--|----|
| Baclofen-mediated suppression of dopamine release is frequency-dependent .   | 67 |
| Baclofen-induced suppression of dopamine release persists in cocaine-sensitised mice.....  | 71 |
| Discussion.....  | 73 |
| Activation of GABA <sub>B</sub> receptors on dopamine terminals suppresses dopamine release in the NAc core .....  | 73 |
| Frequency-dependence of dopamine release .....   | 75 |
| GABA <sub>B</sub> -mediated suppression of dopamine release persists with chronic cocaine treatment.....   | 76 |
| Summary and future directions.....   | 78 |
| Chapter 4 Presynaptic regulation of dopamine release by GABA <sub>B</sub> or D2 receptors in the NAc core is unaltered by access to a cafeteria diet. .... | 79 |
| Introduction .....   | 79 |
| Methods.....   | 81 |
| Cafeteria diet .....   | 81 |
| Fast-scan cyclic voltammetry.....  | 82 |
| Drugs.....   | 84 |
| Statistical analysis .....   | 84 |
| Results.....   | 85 |
| Rats with extended access to a cafeteria diet gained more weight and consumed more calories than restricted access or chow only rats .....                 | 85 |
| GABA <sub>B</sub> receptor modulation of dopamine release is unchanged by access to a cafeteria diet.....  | 89 |
| D2 receptor modulation of dopamine release is unchanged by access to a cafeteria diet.....   | 92 |
| Dopamine re-uptake is unchanged by access to a cafeteria diet.....   | 94 |
| Discussion.....  | 94 |
| GABA <sub>B</sub> receptors on dopamine neuron terminals are resistant to diet-induced alterations .....   | 95 |
| D2 autoreceptor function in the NAc core is intact after diet induced obesity ...  | 97 |
| Dopamine reuptake was not altered by access to a cafeteria diet .....  | 99 |

|   |     |
|---|-----|
| Summary and future directions .....   | 99  |
| Chapter 5 General discussion and conclusions.....   | 101 |
| R-isovaline does not act as a GABA <sub>B</sub> receptor agonist .....  | 101 |
| GABA <sub>B</sub> receptor activation decreases dopamine release in the NAc core of both mice<br>and rats .....   | 104 |
| GABA <sub>B</sub> receptors on dopamine neuron terminals are resistant to changes induced by<br>sensitising cocaine treatment or access to a palatable cafeteria diet. .... | 106 |
| Summary .....   | 108 |
| References .....  | 109 |

**List of tables**

Table 4.1 Nutrient composition of cafeteria diet food..... 82

## List of figures

|  |    |
|--|----|
| Figure 1.1 Schematic diagram of the GABA <sub>B</sub> receptor and effector proteins .....                                 | 3  |
| Figure 1.2 Receptors modulating dopamine release.....  | 27 |
| Figure 2.1 GABA, baclofen and R-isovaline do not evoke currents in untransfected AtT-20 cells.<br>.....                    | 44 |
| Figure 2.2 The GABA <sub>B1</sub> subunit expresses at the membrane in transfected AtT-20 cells.....                       | 45 |
| Figure 2.3 GABA and baclofen, but not isovaline, evoke inwardly rectifying currents in<br>transfected AtT-20 cells.....    | 47 |
| Figure 2.4 R-isovaline does not modulate GABA-evoked currents in transfected AtT-20 cells. ..                              | 49 |
| Figure 2.5 Intracellular R-isovaline does not alter baseline or GABA-mediated currents in<br>transfected AtT-20 cells..... | 50 |
| Figure 2.6 R-isovaline does not activate GABA <sub>B</sub> receptors in cultured hippocampal neurons. ...                  | 52 |
| Figure 3.1 Baclofen suppresses dopamine release evoked by a single pulse stimulus.....                                     | 64 |
| Figure 3.2 Baclofen-mediated suppression of evoked dopamine release does not desensitise<br>over time.....                 | 65 |
| Figure 3.3 Baclofen-mediated suppression of evoked dopamine release does not require<br>cholinergic input.....             | 67 |
| Figure 3.4 Baclofen inhibits dopamine in a frequency dependent manner.....   | 68 |
| Figure 3.5 Baclofen increases the paired pulse ratio of dopamine release.....  | 70 |
| Figure 3.6 Baclofen-mediated suppression of dopamine release persists with sensitising cocaine<br>treatment.....           | 72 |
| Figure 4.1 Increased calorie consumption and weight gain in rats with extended access to a<br>cafeteria diet.....          | 86 |
| Figure 4.2 Rats with extended access to a cafeteria diet have elevated plasma leptin and insulin<br>.....                  | 89 |
| Figure 4.3 GABA <sub>B</sub> modulation of dopamine release in the NAc core is unaltered by cafeteria diet<br>access ..... | 91 |
| Figure 4.4 D2 modulation of dopamine release in the NAc core is unaltered by cafeteria diet<br>access .....                | 93 |
| Figure 4.5 Dopamine reuptake in the NAc core is unaltered by access to a cafeteria diet .....                              | 94 |

## List of abbreviations

|                   |  |
|-------------------|--|
| [DA] <sub>o</sub> | extracellular dopamine concentration                         |
| ACh               | acetylcholine  |
| AMP               | adenosine monophosphate                                      |
| AMPA              | $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid |
| ANOVA             | analysis of variance   |
| ATP               | adenosine triphosphate                                       |
| cAMP              | cyclic adenosine monophosphate                               |
| CNS               | central nervous system                                       |
| DAPI              | 4',6-diamidino-2-phenylindole                                |
| GABA              | $\gamma$ -aminobutyric acid (GABA)                           |
| GDP               | guanosine diphosphate  |
| GFP               | green fluorescent protein                                    |
| GIRK channel      | G-protein coupled inwardly rectifying K <sup>+</sup> channel |
| GPCR              | G-protein coupled receptor                                   |
| G-protein         | guanine nucleotide binding protein                           |
| GTP               | guanosine triphosphate                                       |
| IPSP              | inhibitory post synaptic potential                           |
| KCTD              | K <sup>+</sup> channel tetramerisation domain                |
| mGluR             | metabotropic glutamate receptor                              |
| mPFC              | medial prefrontal cortex                                     |
| mRNA              | messenger ribonucleic acid                                   |

|                  |   |
|------------------|---|
| MSN              | medium spiny neuron                                     |
| NAc              | nucleus accumbens                                       |
| nACh receptor    | nicotinic ACh receptor                                  |
| NMDA             | N-methyl-D-aspartate                                    |
| P1               | pulse 1   |
| P1+P2            | pulse 1 + pulse 2                                       |
| P2               | pulse 2   |
| PKA              | cyclic adenosine monophosphate dependent protein kinase |
| RGS protein      | regulators of G-protein signalling                      |
| Tau              | time constant of decay                                  |
| VFT              | Venus fly trap domain                                   |
| VGCC             | voltage-gated Ca <sup>2+</sup> channel                  |
| V <sub>max</sub> | maximal uptake rate                                     |
| VTA              | ventral tegmental area                                  |

## Acknowledgements

I would like to thank my supervisors Dr. Stephanie Borgland and Dr. Ernest Puil for their guidance throughout my degree. Without their hard work and support I would not have made it to this stage.

I would also like to thank my supervisory committee members Dr. Bernard MacLeod and Dr. Steve Kehl. In addition I would like to thank past chairpersons for my committee meetings Drs. David Fedida, Jim Wright, Dick Wall and Cathy Pang.

Thanks to my past and present lab members for friendship, helpful discussions and scientific and moral support, especially Dr. Jennifer Thompson, Dr. Lindsay Naef, Corey Baimel, Dr. Ryan Whitehead and many others.

Thanks to the graduate student secretary Wynne Leung. Doing the last half of my Ph.D. being away from Vancouver would have been so much more difficult if it wasn't for Wynne always being so willing to help out with and find solutions for all administrative tasks.

Thanks to Dr. David Fedida for cell culturing supplies and expertise, Dr. Shernaz Bamji and Stefano Brigidi for providing cultured hippocampal neurons and Jens-Peter David for help with confocal microscopy.

Thanks to many other friends and colleagues and APT staff not mentioned here but who helped and supported me throughout my Ph.D. and made my time in Vancouver and Calgary the enjoyable experience that it was.

A special thanks to my family for always loving and believing in me and for being OK with me being so far away from home. And finally thanks to Gaith for waking up early to drive me to the lab in the weekend when it was -20 degrees outside, and for picking me up from work when it was late at night (and -20 degrees outside), for making sure I always ate proper food, for letting me drag him on camping trips and long hikes and for always supporting me.

## **Chapter 1 Introduction**

### **Overview and objectives**

This thesis contains 3 manuscripts exploring the effects of ligands for the metabotropic  $\gamma$ -aminobutyric acid (GABA<sub>B</sub>) receptor in heterologous, physiological or pathophysiological circumstances. In the first manuscript I determined whether R-isovaline is a GABA<sub>B</sub> receptor agonist. To do this I created an expression system to test the ability of R-isovaline, in addition to the known GABA<sub>B</sub> receptor agonists, baclofen or GABA, to activate GABA<sub>B</sub> receptor coupled K<sup>+</sup> currents in isolated cells. In the second manuscript I determined mechanisms associated with GABA<sub>B</sub> modulation of dopamine release in the nucleus accumbens (NAc) core. Fast scan cyclic voltammetry in striatal brain slices was employed to measure the actions of baclofen on dopamine release. In the final manuscript I determined whether diet could alter changes in the ability of GABA<sub>B</sub> or dopamine D2 receptors to modulate dopamine release. Two food access regimens were used that induced either excessive weight gain or binge-like eating behaviour. Fast scan cyclic voltammetry in brain slices was again employed to measure the effects of baclofen or the D2 agonist quinpirole on dopamine release in the NAc core.

### **Physiology of GABA<sub>B</sub> receptors**

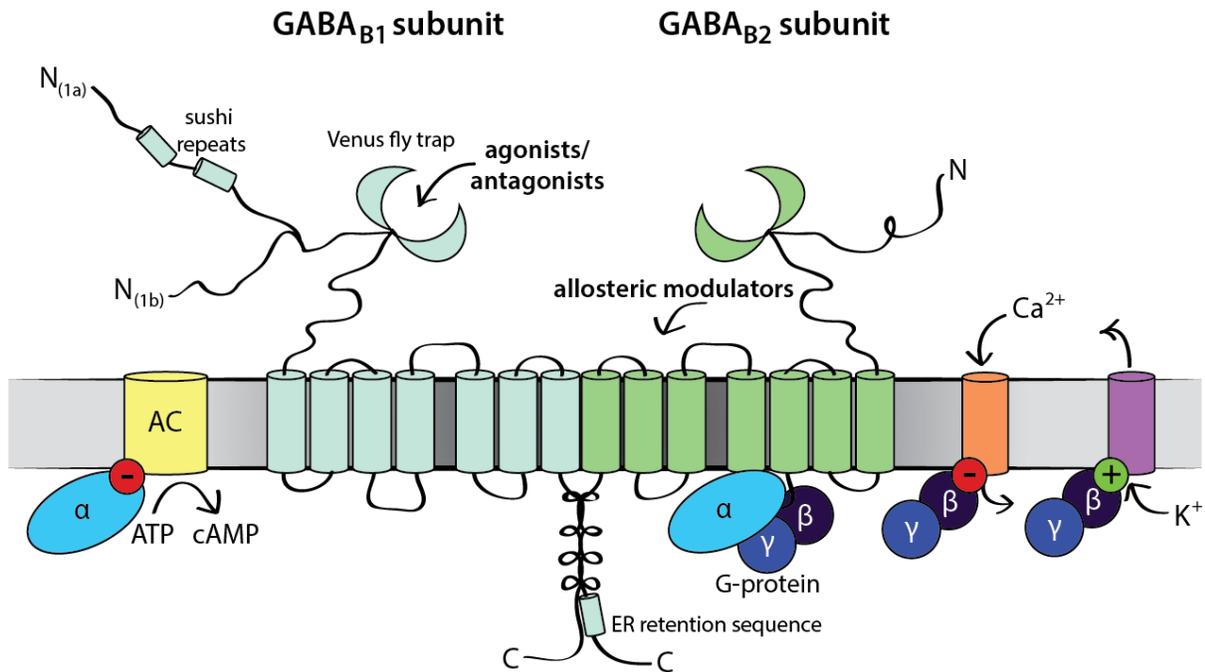
GABA is a major inhibitory neurotransmitter in the central nervous system (CNS) (Krnjević and Schwartz, 1967). There are 2 major receptor types for GABA, the ionotropic GABA<sub>A/C</sub> receptor and the metabotropic GABA<sub>B</sub> receptor. GABA<sub>B</sub> receptors are expressed throughout the CNS and periphery (Bowery et al., 1987; Ong and Kerr, 1990; Bischoff et al., 1999). Agonists of the GABA<sub>B</sub> receptor are reported to have therapeutic potential as muscle relaxants, analgesics, anxiolytics,

and in the pharmacotherapy for substance use (Cutting and Jordan, 1975; Cryan et al., 2004; Dario and Tomei, 2004; Frankowska et al., 2007).

## **Structure**

### **Heterodimerisation**

The GABA<sub>B</sub> receptor belongs to a super family of receptors known as guanine nucleotide binding-protein (G-protein) coupled receptors (GPCRs) (Morishita et al., 1990; Kaupmann et al., 1997). The GABA<sub>B1</sub> subunit was first cloned in 1997 (Kaupmann et al., 1997). However, expression of this subunit in heterologous cells did not replicate responses observed at GABA<sub>B</sub> receptors in native receptor preparations. Subsequently, 3 groups independently determined that this was because the GABA<sub>B</sub> receptor is an obligate heterodimer (Jones et al., 1998a; Kaupmann et al., 1998; White et al., 1998, Figure 1.1). Both a GABA<sub>B1</sub> and a GABA<sub>B2</sub> subunit are required for a functional GABA<sub>B</sub> receptor (Jones et al., 1998a; Kaupmann et al., 1998; White et al., 1998; Kuner et al., 1999). The GABA<sub>B1</sub> subunit contains an endoplasmic reticulum retention sequence on the C-terminal tail that renders it unable to traffic to the cell surface on its own (Couve et al., 1998). Association of the C-terminal tails of the GABA<sub>B1</sub> and GABA<sub>B2</sub> subunits masks this retention sequence, enabling cell surface expression (White et al., 1998; Pagano et al., 2001).



**Figure 1.1 Schematic diagram of the GABA<sub>B</sub> receptor and effector proteins**

The GABA<sub>B</sub> receptor is an obligate heterodimer made up of a GABA<sub>B1</sub> and GABA<sub>B2</sub> subunit. The GABA<sub>B1</sub> subunit contains an endoplasmic reticulum (ER) retention sequence on the C-terminal tail that prevents cell surface trafficking. There are two isoforms of the GABA<sub>B1</sub> subunit termed GABA<sub>B1a</sub> and GABA<sub>B1b</sub>. They differ by the addition of sushi repeat domains on the N-terminal tail of the GABA<sub>B1a</sub> isoform. Agonists and antagonists of the GABA<sub>B</sub> receptor bind within a site on the GABA<sub>B1</sub> subunit known as a Venus fly trap domain. Allosteric modulators interact with the transmembrane domain of the GABA<sub>B2</sub> subunit. The GABA<sub>B2</sub> subunit couples to the G-protein. Typical cellular effects of GABA<sub>B</sub> receptor activation include G<sub>α</sub>-mediated inhibition of adenylate cyclase (AC), and G<sub>βγ</sub>-mediated inhibition of voltage gated Ca<sup>2+</sup> channels and activation of G-protein coupled inwardly rectifying K<sup>+</sup> channels. Adapted from Bowery and Smart, 2006 with permission.

Both subunits of the GABA<sub>B</sub> receptor contain an extracellular N-terminus domain, 7-transmembrane (heptahelical) domain and intracellular C-terminal domain (Kaupmann et al., 1997; Geng et al., 2013), however, GPCRs actions are split between the 2 subunits. The GABA<sub>B1</sub> subunit contains the orthosteric ligand binding site (Kaupmann et al., 1997; Galvez et al., 2000a;

Margeta-Mitrovic et al., 2001b; Geng et al., 2013) while the intracellular loops of the heptahelical domain of the GABA<sub>B2</sub> subunit are responsible for G-protein coupling (Margeta-Mitrovic et al., 2001a; Robbins et al., 2001; Duthey et al., 2002; Havlickova et al., 2002). The GABA<sub>B2</sub> subunit allosterically enhances ligand binding at the GABA<sub>B1</sub> subunit and the GABA<sub>B1</sub> subunit allosterically enhances GABA<sub>B2</sub>-mediated G-protein coupling (Galvez et al., 2001; Margeta-Mitrovic et al., 2001b; Geng et al., 2013).

Interestingly, the expression of GABA<sub>B1</sub> and GABA<sub>B2</sub> messenger ribonucleic acid (mRNA) is not completely overlapping. In some brain areas including the striatum there is differential expression of both mRNA and protein for the GABA<sub>B1</sub> and GABA<sub>B2</sub> subunits (Clark et al., 2000; Ng and Yung, 2001; Martin et al., 2004). There is evidence that GABA<sub>B</sub> receptor subunits can associate with other GPCRs (Chang et al., 2007; Cheng et al., 2007; Boyer et al., 2009). However, typical GABA<sub>B</sub> receptor functions are lost with knockout of either GABA<sub>B</sub> receptor subunit in mice (Prosser et al., 2001; Schuler et al., 2001; Quéva et al., 2003; Gassmann et al., 2004).

Atypical baclofen-mediated inhibition of K<sup>+</sup> channels has been observed in CA1 pyramidal cells in GABA<sub>B2</sub> knockout mice (Gassmann et al., 2004). One hypothesis is that excessive accumulation of the GABA<sub>B1</sub> subunit in the endoplasmic reticulum may overload endoplasmic reticulum retention mechanisms resulting in a small proportion of GABA<sub>B1</sub> subunits trafficking to the cell membrane (Gassmann et al., 2004). The physiological relevance of this is unknown and the consensus is that both subunits are required for typical GABA<sub>B</sub> mediated effects.

## **Isoforms**

There is surprisingly little molecular diversity in GABA<sub>B</sub> receptors. The GABA<sub>B2</sub> subunit has no isoforms. There are 2 functionally expressed isoforms of the GABA<sub>B1</sub> subunit, termed GABA<sub>B1a</sub> and GABA<sub>B1b</sub> (Kaupmann et al., 1997). The 2 GABA<sub>B1</sub> isoforms result from alternative promoter sites on the same gene (Steiger et al., 2004). The GABA<sub>B1a</sub> subunit contains a pair of short consensus repeats, also known as complement control proteins or sushi domains (Hawrot et al., 1998; Blein et al., 2004). The sushi domains serve as an axonal targeting sequence for the GABA<sub>B1a</sub>-containing GABA<sub>B</sub> receptor (Vigot et al., 2006; Biermann et al., 2010). They have no impact on ligand binding or receptor function and when expressed in heterologous expression systems there are no pharmacological or functional differences between GABA<sub>B</sub> receptors containing the GABA<sub>B1a</sub> or GABA<sub>B1b</sub> isoforms (Waldmeier et al., 1994; Bräuner-Osborne and Krogsgaard-Larsen, 1999). Other GABA<sub>B1</sub> isoforms have been proposed but little evidence has been presented as to their functional consequences (Isomoto et al., 1998; Pfaff et al., 1999; Wei et al., 2001a, 2001b; Tiao et al., 2008).

## **Orthosteric ligand binding**

The orthosteric binding site of the GABA<sub>B</sub> receptor is located on the extracellular N-terminus of the GABA<sub>B1</sub> subunit where critical residues bind both the carboxylate and amino groups of agonists (Galvez et al., 2000a; Geng et al., 2013). The binding site is modeled as a Venus fly trap (VFT), comprising 2 lobes and a hinge. Crystal structures of the GABA<sub>B</sub> receptor have shown that in the resting (unbound) state the lobes of the GABA<sub>B1</sub> subunit VFT are in an open conformation (Geng et al., 2013). Agonists interact with both lobes of the GABA<sub>B1</sub> VFT to

stabilise the closure of the 2 lobes (Geng et al., 2013). Antagonist binding prevents the closure of the VFT lobes (Geng et al., 2013).

While the GABA<sub>B2</sub> subunit does not bind orthosteric ligands it increases the affinity of the GABA<sub>B</sub> receptor for agonists by interactions between the GABA<sub>B1</sub> and GABA<sub>B2</sub> ectodomains (Galvez et al., 2001; Liu et al., 2004; Nomura et al., 2008; Geng et al., 2012). The GABA<sub>B2</sub> subunit also contains a VFT comprised of 2 lobes, however, the VFT of the GABA<sub>B2</sub> subunit remains in the open conformation whether ligands are bound or not (Geng et al., 2013). Extracellular Ca<sup>2+</sup> concentration can alter the affinity of GABA for the GABA<sub>B</sub> receptor, but has no effect on R-baclofen binding (Galvez et al., 2000b). Ca<sup>2+</sup> is thought to interact within the GABA<sub>B1</sub> VFT to help stabilise the closed conformation in the presence of GABA (Galvez et al., 2000b).

## **Function**

### **Subcellular localisation**

The role of the GABA<sub>B</sub> receptor in synaptic signalling depends upon subcellular localisation of the GABA<sub>B</sub> receptor. GABA<sub>B</sub> receptors can function as both autoreceptors (on GABA neurons) or as heteroreceptors (on non-GABA neurons) and can be localised both pre and post-synaptically.

Due to the sushi domains of the GABA<sub>B1a</sub> isoform acting as axonal targeting sequences the GABA<sub>B1a-B2</sub> receptors are more likely to be expressed at presynaptic sites whereas GABA<sub>B1b-B2</sub> receptors are more often found postsynaptically (Vigot et al., 2006; Guetg et al., 2009; Biermann et al., 2010).

The majority of GABA<sub>B</sub> receptors are located extra- synaptically and very rarely are they found within the synapse active zone or on postsynaptic densities (Scanziani, 2000; Kulik et al., 2003;

Luján et al., 2004; Lacey et al., 2005). Due to the extrasynaptic localisation and efficient GABA uptake mechanisms strong stimulations are needed to release sufficient GABA to activate GABA<sub>B</sub> receptors (Isaacson et al., 1993; Scanziani, 2000; Kulik et al., 2003). Such release could occur with coordinated activity of neurons or, alternatively, with activity dependent release of GABA from dendrites or glial cells (Zilberter et al., 1999; Scanziani, 2000; Angulo et al., 2008; Velez-Fort et al., 2012).

### **Signalling via G<sub>α</sub> and G<sub>βγ</sub>**

When a GPCR is activated by an agonist it induces a conformational change in the receptor protein which catalyses the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) in the associated G-protein. This enables dissociation of the heterotrimeric G-protein into G<sub>α</sub>- and G<sub>βγ</sub>-subunits. The action of a GPCR is terminated when the GTP bound to the G<sub>α</sub>- subunit is hydrolysed back into GDP, facilitating reassociation of the G<sub>α</sub>- and G<sub>βγ</sub>-subunits, enabling reassociation of the complete G-protein with a GPCR (Swillens et al., 1979; Kwok-Keung Fung and Stryer, 1980; Stadel et al., 1981; Deterre et al., 1984; Blumer and Thorner, 1990). GABA<sub>B</sub> receptors couple to the inhibitory, pertussis toxin sensitive, G<sub>i/o</sub> G-protein (Hill, 1985; Xu and Wojcik, 1986; Morishita et al., 1990). Conventional cellular effects include slow and long lasting actions through G<sub>α<sub>i/o</sub></sub>- mediated inhibition of adenylate cyclase and more rapid actions through G<sub>βγ</sub>- mediated activation of G-protein coupled inwardly rectifying K<sup>+</sup> (GIRK) channels and inhibition of voltage-gated Ca<sup>2+</sup> channels (VGCCs) (Padgett and Slesinger, 2010).

## **Adenylate cyclase**

Like other GPCRs that couple to the inhibitory  $G_{i/o}$  G-protein, GABA<sub>B</sub> receptor activation inhibits adenylate cyclase. Adenylate cyclase catalyses the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). As a consequence, GABA<sub>B</sub> receptor activation can inhibit the production of cAMP (Hill, 1985; Xu and Wojcik, 1986). The downstream consequences of GABA<sub>B</sub> signalling through  $G_{\alpha i/o}$  are not well understood but have been shown to include modulation of ion channels including N-methyl-D-aspartate (NMDA) receptors (Chalifoux and Carter, 2010), A-type  $K^+$  channels (Saint et al., 1990), 2-pore domain mechanosensitive  $K^+$  channels (Deng et al., 2009), as well as transcription factors (White et al., 2000; Ren and Mody, 2006), intracellular  $Ca^{2+}$  signalling cascades (New et al., 2006), and kinase activity (Diversé-Pierluissi et al., 1997; Couve et al., 2002; Ren and Mody, 2003). Through  $G_{\alpha}$ -mediated signalling, GABA<sub>B</sub> receptor activation can decrease the surface stability of GABA<sub>B</sub> receptors in a cAMP dependent protein kinase (PKA)-dependent fashion (Couve et al., 2002) and inhibit neurotransmitter release by retarding synaptic vesicle recruitment (Sakaba and Neher, 2003).

## **GIRK channels**

GIRK channels, also known as Kir3 channels, are members of the inwardly rectifying  $K^+$  channel family that are activated by  $G_{\beta\gamma}$  subunits specifically from  $G_{i/o}$  G-proteins (Kofuji et al., 1995; Rubinstein et al., 2007). GABA<sub>B</sub> receptor mediated activation of GIRK channels occurs by a rapid, membrane delimited action, assisted by the GABA<sub>B</sub> receptor and GIRK channel existing at the membrane as a preformed complex with the G-protein (Sadjja et al., 2003; David et al., 2006; Doupnik, 2008; Ciruela et al., 2010). Coupling of GIRK channels to GABA<sub>B</sub> receptors most commonly occurs at postsynaptic sites, although can also occur presynaptically (Lüscher et al.,

1997; Ladera et al., 2008; Fernández-Alacid et al., 2009). Postsynaptic GABA<sub>B</sub>-GIRK receptor activation hyperpolarises the membrane inhibiting neuron excitability and is responsible for the slow component of the inhibitory postsynaptic potential (IPSP) (Isaacson et al., 1993; Lüscher et al., 1997).

GIRK channels are tetramers comprised of Kir3.1 - 3.4 subunits (Kubo et al., 1993; Lesage et al., 1994; Kofuji et al., 1995). The most common subunit composition in the brain is a heteromultimer containing 2 different subunits (Kofuji et al., 1995; Slesinger et al., 1997; Uezono et al., 1998; Jelacic et al., 2000). In dopamine neurons of the ventral tegmental area (VTA) it has been shown that the subunit composition can affect channel properties which in turn can alter the EC<sub>50</sub> of ligands acting at GPCRs-coupled to GIRK channels (Cruz et al., 2004; Labouèbe et al., 2007).

In Chapter 2 I measured the actions of GABA<sub>B</sub> agonists at GABA<sub>B</sub> receptors coupled to GIRK channels. I heterologously expressed GABA<sub>B</sub> receptors in mouse pituitary AtT-20 cells which endogenously express GIRK channels (Dousmanis and Pennefather, 1992). I also examined the actions of GABA<sub>B</sub> agonists in hippocampal pyramidal neurons which have previously been shown to express GABA<sub>B</sub>-GIRK receptors (Gähwiler and Brown, 1985; Sodickson and Bean, 1996; Leaney, 2003).

### **VGCCs**

Coupling between VGCCs and GABA<sub>B</sub> receptors typically occurs at presynaptic sites and is responsible for inhibition of Ca<sup>2+</sup> dependent neurotransmitter release (Dunlap and Fischbach, 1981; Robertson and Taylor, 1986; Mintz and Bean, 1993; Huston et al., 1995; Yamada et al.,

1999). GABA<sub>B</sub> receptors have been shown to modulate Ca<sup>2+</sup> currents through VGCCs in acutely isolated midbrain dopamine neurons (Cardozo and Bean, 1995). The GABA<sub>B</sub> receptor traditionally couples to the N- and P/Q-type VGCCs which are the predominant VGCCs regulating neurotransmitter release in the CNS (Menon-Johansson et al., 1993; Mintz and Bean, 1993; Guyon and Leresche, 1995; Huston et al., 1995; Chen and van den Pol, 1998; Harayama et al., 1998; Bussi eres and El Manira, 1999; Barral et al., 2000).

GPCR-inhibition of VGCCs is mediated via the G<sub>βγ</sub> subunit (Herlitze et al., 1996; Delmas et al., 1998). G<sub>βγ</sub> binding slows the activation kinetics of channel opening and alters the voltage dependence of the VGCC so that a greater depolarisation of the nerve terminal is required for the VGCC to open (Bean, 1989; Herlitze et al., 1996). Similar to GABA<sub>B</sub> receptor coupling to GIRK channels, inhibition of VGCCs by GABA<sub>B</sub> receptor activation is thought to occur by a rapid membrane delimited action due to preformed complexes of GABA<sub>B</sub> receptors with VGCCs (Laviv et al., 2011).

### **Regulation of GABA<sub>B</sub> responses**

Many studies in native receptor preparations have reported diversity in the sensitivity of GABA<sub>B</sub> receptors to ligands and variation in GABA<sub>B</sub> receptor-mediated responses (Colmers and Williams, 1988; Dutar and Nicoll, 1988; Bonanno and Raiteri, 1993; Deisz et al., 1993; Fassio et al., 1994; Guyon and Leresche, 1995; Cunningham and Enna, 1996; Bonanno et al., 1997; Pozza et al., 1999; Yu et al., 1999; Cruz et al., 2004; Li and Stern, 2004). However no confirmable differences in the rank order of ligand potencies have been reported supporting the lack of

pharmacologically distinct subtypes (Bräuner-Osborne and Krogsgaard-Larsen, 1999; Pinard et al., 2010).

Instead, differences in GABA<sub>B</sub> receptor mediated responses are thought to be regulated by the membrane expression of the GABA<sub>B</sub> receptors or effector channels, compartmentalisation and/or coupling efficacy between GABA<sub>B</sub> receptors and their effector channels, and the kinetics of effector channel activation and desensitisation. These have been shown to be under the influence of a variety of cell specific proteins including the GABA<sub>B</sub> auxiliary subunits (Schwenk et al., 2010), regulators of G-protein signalling (RGS) proteins (Labouèbe et al., 2007), as well as kinases (Taniyama et al., 1991; Couve et al., 2002; Perroy et al., 2003; Pontier et al., 2006; Kanaide et al., 2007; Kuramoto et al., 2007; Adelfinger et al., 2014).

### **Cell surface expression**

GABA<sub>B</sub> receptors undergo rapid constitutive internalisation and recycling back to the membrane (Grampp et al., 2007, 2008; Vargas et al., 2008; Wilkins et al., 2008). The 2 GABA<sub>B</sub> subunits internalise as a dimer with the GABA<sub>B2</sub> subunit controlling the rate of internalisation (Hannan et al., 2011). Phosphorylation of the GABA<sub>B2</sub> subunit by PKA or AMP dependent kinase stabilises the GABA<sub>B</sub> receptor at the cell membrane, whereas a decrease in GABA<sub>B2</sub> subunit phosphorylation is associated with a reduction in the cell surface expression of GABA<sub>B</sub> receptors and reduced effects of GABA<sub>B</sub> agonists (Couve et al., 2002; Xi et al., 2003; Fairfax et al., 2004; Kuramoto et al., 2007; Terunuma et al., 2010; Padgett et al., 2012; Hearing et al., 2013). In Chapter 3 I examined whether prolonged activation of GABA<sub>B</sub> receptors desensitised GABA<sub>B</sub> mediated modulation of dopamine release in the NAc.

GABA<sub>B</sub> receptor cell surface expression can also be altered by targeting internalised GABA<sub>B</sub> receptors to lysosomal degradation pathways instead of recycling pathways which reduces the number of available GABA<sub>B</sub> receptors. It has been shown that activation of glutamate receptors including  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), NMDA and group 1 metabotropic glutamate receptors (mGluRs), can alter the proportion of GABA<sub>B</sub> receptors sent to degradation pathways without affecting the rate of GABA<sub>B</sub> receptor internalisation (Vargas et al., 2008; Maier et al., 2010; Terunuma et al., 2010).

### **Auxiliary subunits**

4 members of the K<sup>+</sup> channel tetramerisation domain (KCTD) family (KCTD 8, 12, 12b and 16) act as GABA<sub>B</sub> receptor auxiliary subunits (Schwenk et al., 2010). These KCTD proteins are expressed widely throughout the CNS including in the hippocampus, VTA and NAc (Metz et al., 2011). Association of a KCTD protein-tetramer and a GABA<sub>B</sub> receptor occurs within a region on the intracellular C-terminal domain of the GABA<sub>B2</sub> subunit. KCTD proteins stabilise the GABA<sub>B</sub> receptor at the cell membrane, reducing constitutive internalisation and can modulate agonist potency, kinetics and desensitisation of GABA<sub>B</sub> responses (Schwenk et al., 2010; Ivankova et al., 2013; Turecek et al., 2014). K<sup>+</sup> currents induced by GABA<sub>B</sub> receptors associated with KCTD 12, but not KCTD 8 or 16, rapidly desensitise as KCTD 12 binds G <sub>$\beta\gamma$</sub> , preventing G <sub>$\beta\gamma$</sub>  association with GIRK channels (Schwenk et al., 2010; Seddik et al., 2012; Turecek et al., 2014). Phosphorylation of the GABA<sub>B</sub> receptor by PKA slows the rate of KCTD 12 mediated desensitisation, whereas inhibition of PKA, as would be the case after prolonged GABA<sub>B</sub> receptor activation, accelerates KCTD 12 mediated rapid desensitisation (Adelfinger et al., 2014).

KCTD proteins can also differentially modify agonist potency. In Chinese hamster ovary cells heterologously expressing GABA<sub>B</sub> receptors and either KCTD 12 or 16, the EC<sub>50</sub> of baclofen at GABA<sub>B</sub> receptors was decreased 7 or 3 fold, respectively, compared with no KCTD protein (Schwenk et al., 2010), and KCTD 8 and 16 increased the affinity of GABA for the GABA<sub>B</sub> receptor in transfected human embryonic kidney 293 cells (Rajalu et al., 2015).

### **RGS proteins**

RGS proteins also play an important role in regulating the signalling kinetics and efficacy of GABA<sub>B</sub> receptor coupling (Mutneja et al., 2005; Fowler et al., 2007; Labouèbe et al., 2007). RGS proteins belong to a family of GTPase-accelerating proteins (Berman et al., 1996; Watson et al., 1996). They promote the G<sub>α</sub>-mediated hydrolysis of GTP to GDP and consequently accelerate the termination of GABA<sub>B</sub>-receptor mediated responses (Berman et al., 1996; Hunt et al., 1996; Watson et al., 1996; Doupnik et al., 1997). RGS 2 and 4 regulate GABA<sub>B</sub> receptor signalling in the VTA while RGS 7 is involved in regulating GABA<sub>B</sub> mediated responses in hippocampal pyramidal neurons (Fowler et al., 2007; Labouèbe et al., 2007; Xie et al., 2010; Fajardo-Serrano et al., 2013; Ostrovskaya et al., 2014). Modulating the expression of RGS proteins can alter GABA<sub>B</sub> receptor kinetics and coupling efficiency, altering the EC<sub>50</sub> for ligand actions at the GABA<sub>B</sub> receptor in the VTA or hippocampus (Labouèbe et al., 2007; Ostrovskaya et al., 2014).

## **Pharmacology of GABA<sub>B</sub> receptors**

### **Baclofen**

In the research undertaken in Chapters 2-4 I used the R-enantiomer of baclofen, a full and selective GABA<sub>B</sub> receptor agonist, to examine the effects of GABA<sub>B</sub> receptor activation. Baclofen

is the prototypical GABA<sub>B</sub> receptor agonist. It was synthesised in 1962 in an attempt to make GABA more lipophilic to increase its blood brain barrier permeability (reviewed by Froestl, 2010). Unlike GABA, baclofen does cross the blood brain barrier, although it has been determined that this is due to active transport by an amino acid transporter (van Bree et al., 1988; van Bree et al., 1991). The R-enantiomer of baclofen has significantly higher activity at the GABA<sub>B</sub> receptor than the S-enantiomer (Froestl et al., 1995). Baclofen is licenced for use as a muscle relaxant for patients with spasticity, and was an integral part in the discovery of the GABA<sub>B</sub> receptor (Brogden et al., 1974; Bowery and Hudson, 1979; Bowery et al., 1979; Brown and Higgins, 1979; Hill and Bowery, 1981).

Therapeutic use of baclofen is limited due to the development of side effects such as sedation, muscle weakness, deficits in cognition, hypothermia and exacerbation of depression (Sawa and Paty, 1979; Nakagawa et al., 1996; Quéva et al., 2003; van Nieuwenhuijzen and McGregor, 2009; Whitehead et al., 2012). Tolerance to the therapeutic effects of baclofen also develops rapidly and withdrawal can be significant (Garabedian-Ruffalo and Ruffalo, 1985; Dario and Tomei, 2004). Consequently, alternative approaches are needed in order to further utilise the GABA<sub>B</sub> receptor as a therapeutic target.

### **Allosteric modulation of the GABA<sub>B</sub> receptor**

One such alternative approach is the use of positive allosteric modulators of the GABA<sub>B</sub> receptor. Allosteric modulators act at sites other than the orthosteric ligand binding site. They do not activate the receptor when applied alone but can alter the effect of agonists or antagonists and amplify the effect of endogenous neurotransmitter release. Positive allosteric

modulators of the GABA<sub>B</sub> receptor, such as CGP7930 or GS39783, increase both the efficacy and potency of the GABA<sub>B</sub>-receptor mediated response by interacting with the heptahelical domain of the GABA<sub>B2</sub> subunit (Urwyler et al., 2001, 2003; Binet et al., 2004; Dupuis et al., 2006).

Current research suggests that allosteric modulators do not produce adverse side effects to the same extent as GABA<sub>B</sub> agonists but can show promising therapeutic potential (Cryan et al., 2004; Smith et al., 2004; Orrù et al., 2005; Liang et al., 2006; Filip et al., 2007; Maccioni et al., 2007, 2008).

### **Isovaline, an atypical GABA<sub>B</sub> agonist**

Isovaline is an unusual non-proteinogenic  $\alpha$ -amino acid. In animal models isovaline has been shown to have analgesic, anti-convulsant and anxiolytic properties (MacLeod et al., 2010; Whitehead et al., 2012; Sase et al., 2013; Yu et al., 2014; Asseri et al., 2015). Notably, isovaline does not appear to induce the side effects typical of GABA<sub>B</sub> receptor agonists (Whitehead et al., 2012). It is thought that this is due to the inability of isovaline to cross the blood brain barrier (Shiba et al., 1989; Whitehead et al., 2012). However, other studies have reported central effects after peripheral administration of isovaline, suggesting that isovaline may cross the blood brain barrier in certain situations such as rodent models of epilepsy (Sase et al., 2013; Yu et al., 2014). Studies on the analgesic effect of isovaline have shown that it is mediated in part by activation of peripheral GABA<sub>B</sub> receptors (Whitehead et al., 2012). More recently, it has been shown that antagonism of peripheral group 2 mGluRs, which couple to the same inhibitory family of G-proteins as GABA<sub>B</sub> receptors, can also attenuate isovaline induced analgesia (Asseri et al., 2015).

Electrophysiological studies in thalamic brain slices also demonstrated participation of GABA<sub>B</sub> receptors in the mechanism of action of R-isovaline (Cooke et al., 2012). R-isovaline increased the membrane conductance for K<sup>+</sup> in a proportion of neurons tested (Cooke et al., 2009, 2012). This was shown to be dependent upon G-protein action as intracellular application of the non-functional GTP analogues, GDP-β-S and GTP-γ-S, prevented R-isovaline action. Pre-applications of GABA<sub>B</sub> receptor antagonists (CGP 35348 or CGP 52432) were able to prevent the R-isovaline-induced conductance change while antagonists of μ-opioid and muscarinic acetylcholine (ACh) receptors, which can also couple to K<sup>+</sup> channels, were without effect. The GABA<sub>B</sub> receptor positive allosteric modulator, CGP 7930, enhanced the percent conductance change induced by a low concentration of R-isovaline (10 μM) (Cooke et al., 2012).

An indirect effect of R-isovaline on GABA<sub>B</sub>-receptor mediated K<sup>+</sup> currents by increasing extracellular GABA was ruled out for a variety of reasons. Firstly, R-isovaline had no effect on the membrane conductance for Cl<sup>-</sup> and a GABA<sub>A</sub> receptor antagonist had no effect on R-isovaline mediated responses (Cooke, 2010). Secondly, the effect of GABA at the GABA<sub>B</sub> receptor is dependent upon extracellular Ca<sup>2+</sup> levels, while changing extracellular Ca<sup>2+</sup> had no effect on R-isovaline mediated responses (Cooke et al., 2012). Finally, the effect of R-isovaline was blocked by pre-application of GABA<sub>B</sub> receptor agonists but not by an application once an effect had been initiated, which would not have been the case if it was a GABA mediated effect (Cooke et al., 2012).

Baclofen (R-enantiomer) also induced an increase in the membrane conductance for K<sup>+</sup>, similar to R-isovaline, however, there were important differences between the response to baclofen

and R-isovaline (Cooke et al., 2012). Firstly, the time courses of R-isovaline and baclofen action were markedly different. The effects of baclofen were fast in onset and reversed rapidly upon wash off while effects of R-isovaline were slow in onset and long lasting (Cooke et al., 2009, 2012). The proportion of neurons tested that responded to R-isovaline (70%) was significantly less than that that responded to baclofen (94%) and accordingly, some baclofen responsive neurons did not respond to R-isovaline. In addition, in neurons that responded to both R-isovaline and baclofen, co-application resulted in sub-additive conductance changes (Cooke et al., 2012). Interestingly, the concentration-response curve for the R-isovaline induced conductance increase was non-sigmoidal, tending to decrease at higher concentrations. Very high concentrations (1 mM) often had no effect on membrane conductance (Cooke et al., 2009). These discrepancies warranted further investigation into the action of R-isovaline at the GABA<sub>B</sub> receptor. In Chapter 2 I assessed R-isovaline action at GABA<sub>B</sub> receptors in mouse pituitary AtT-20 cells and cultured hippocampal neurons in comparison to known GABA<sub>B</sub> receptor agonists.

### **GABA<sub>B</sub> modulation of drug self-administration and rewarding effects of drugs of abuse**

Enhancing GABA<sub>B</sub> receptor activity, either through application of GABA<sub>B</sub> agonists or positive allosteric modulators, decreases self-administration of a wide range of stimulant and depressant drugs of abuse including amphetamine (Brebner et al., 2005a), cocaine (Roberts and Andrews, 1997; Shoaib et al., 1998; Brebner et al., 1999, 2000a; Campbell et al., 1999; Smith et al., 2004; Filip et al., 2007), nicotine (Fattore et al., 2002; Corrigall et al., 2000; Paterson et al., 2004, 2005, 2008), heroin (Xi and Stein, 1999) and ethanol (Colombo et al., 2000; Daoust et al.;

Anstrom et al., 2003; Maccioni et al., 2005, 2007; Orrù et al., 2005; Liang et al., 2006; Walker and Koob, 2007). Experiments using a progressive ratio schedule of drug delivery, where the number of responses required to obtain a drug increases after each drug delivery, suggest that GABA<sub>B</sub> agonists or positive allosteric modulators can decrease the point at which animals stop responding for drugs (Roberts et al., 1996; Brebner et al., 1999, 2000a, 2000b, 2005a; Ranaldi and Poeggel, 2002; Paterson et al., 2004, 2008; Smith et al., 2004; Walker and Koob, 2007; Maccioni et al., 2008). This indicates that GABA<sub>B</sub> receptor activation can decrease the motivation to obtain drugs of abuse (Hodos, 1961).

Activation of GABA<sub>B</sub> receptors can also decrease the rewarding effect of electrical stimulation of brain reward pathways. Baclofen and other GABA<sub>B</sub> receptor agonists have been shown to decrease responding for intracranial self-stimulation (ICSS) and increase the ICSS threshold (Fenton and Liebman, 1982; Willick and Kokkinidis, 1995; Macey et al., 2001; Panagis and Kastellakis, 2002; Paterson et al., 2008). In ICSS tests animals electrically stimulate brain areas/pathways involved in mediating reward (Carlezon and Chartoff, 2007; Negus and Miller, 2014). The ICSS threshold can either be defined as the stimulation frequency at which animals start to respond, suggesting that the animals perceive that stimulation as rewarding, or it can be defined as the minimum stimulation frequency required to maintain responding at a defined rate (Carlezon and Chartoff, 2007; Negus and Miller, 2014). An increase in the ICSS threshold, as observed after baclofen, indicates that a higher frequency electrical stimulation is needed before the animals perceive the stimulation as rewarding. In addition, baclofen has been shown to prevent decreases in the ICSS mediated by cocaine or nicotine (Dobrovitsky et al., 2002; Slattery et al., 2005; Paterson et al., 2008).

Conditioned place preference is another behavioural indicator of the rewarding effects of drugs of abuse. In this test animals are trained to associate one environment with drug administration while another environment is paired with vehicle administration. On the test day drug free animals are allowed access to both environments and the preference is measured for the drug-paired environment. GABA<sub>B</sub> agonists or positive allosteric modulators suppress the development and/or the expression of conditioned place preference for morphine (Tsuji et al., 1996; Kaplan et al., 2003; Sahraei et al., 2009), ethanol (Bechtholt and Cunningham, 2005), nicotine (Mombereau et al., 2007; Le Foll et al., 2008), and amphetamine (Li et al., 2001).

Activation of GABA<sub>B</sub> receptors can also attenuate drug induced increases in locomotor activity. Increased locomotor activity involves activation of the mesolimbic dopamine pathway (Filip and Siwanowicz; Hedou et al., 1999; Heidbreder et al., 1999) and has been proposed as a marker of the “euphoria” or rewarding properties induced by drugs of abuse (Wise and Bozarth, 1987).

Activation of GABA<sub>B</sub> receptors by baclofen inhibits the acute effect of cocaine (Frankowska et al., 2009) and nicotine (Le Foll et al., 2008) on locomotor activity. When drugs are given repeatedly over time on an intermittent schedule an increase in the locomotor activity induced by acute drug administration is often observed. The enhanced drug induced-locomotor activity can exist for days after the last dose of drug and is referred to as locomotor sensitisation. The expression of locomotor sensitisation is a behavioural indicator of drug-induced neuro-adaptations and is indicative of increased reinforcement from, and motivation for, drugs of abuse (Robinson and Berridge, 1993; Brebner et al., 2005b; Steketee and Kalivas, 2011).

Systemic administration of baclofen has been shown to prevent both the development and expression of locomotor sensitisation to cocaine (Frankowska et al., 2009). In Chapter 3 I used a

regimen of cocaine administration that produced locomotor sensitisation in order to determine if there were alterations in GABA<sub>B</sub> regulation of dopamine release.

Consistent with the preclinical evidence reported above, results from a clinical trial support more research into baclofen as a treatment for cocaine addiction (Shoptaw et al., 2003). In a double-blind placebo controlled study of chronic cocaine users participants assigned to receive baclofen were more likely to provide cocaine metabolite-free urine samples than those assigned to receive placebo. Baclofen also decreased activation of brain reward pathways induced by subliminal cues predicting cocaine administration in cocaine-dependent participants (Young et al., 2014).

### **GABA<sub>B</sub> regulation of food intake**

The role of GABA<sub>B</sub> receptors in modulating food intake is complex with many studies reporting contradictory results. There are studies reporting that systemic or intracerebroventricular administration of baclofen can decrease (Zarrindast et al., 1989; Anstrom et al., 2003; Buda-Levin et al., 2005; Filip et al., 2007; Le Foll et al., 2008), have no effect on (Roberts and Andrews, 1997) or even increase (Ebenezer, 1990; Ebenezer and Pringle, 1992; Higgs and Barber, 2004; Bains and Ebenezer, 2013) food consumption or responding for food rewards. Oftentimes responding for food rewards is measured as a control to assess whether behavioural effects of baclofen are due to general disruption of behaviour, which could imply that any effects are primarily due to sedation (Roberts and Andrews, 1997; Anstrom et al., 2003; Filip et al., 2007; Le Foll et al., 2008; Maccioni et al., 2008; Paterson et al., 2008).

Contrasting results could be explained in part by either the macronutrient composition of food and/or the schedule of availability of palatable food. In animal models a binge pattern of feeding is defined as higher than normal food consumption within a restricted time period. Baclofen inhibited binge consumption of vegetable shortening (Buda-Levin et al., 2005; Berner et al., 2009) but did not reduce shortening consumption in a group of rats that were given extended access to shortening, suggesting that baclofen may primarily affect food intake when there is restricted access to that food (Buda-Levin et al., 2005). Baclofen is also less effective at inhibiting binge consumption of sugar than fat (Berner et al., 2009; Wong et al., 2009; although see Anstrom et al., 2003; Maccioni et al., 2005). Baclofen inhibited binge consumption of a 3.2% or a 10% sugar:fat mixture but had no effect on a sugar:fat mixture containing 32% sugar (Wong et al., 2009).

The reasons why baclofen has effects in some feeding regimens or on some food types, but not others is unclear. It is possible that more highly palatable sugar or sugar/fat combinations may override baclofen's ability to inhibit food intake (Wong et al., 2009). Alternatively the mechanisms controlling sugar and fat intake may be differentially regulated by GABA<sub>B</sub> receptors (Berner et al., 2009). It has also been suggested that baclofen may be more efficacious in inhibiting binge consumption of fat because the brain pathways regulating binge-consumption may be more similar to the brain pathways activated by drugs of abuse (Buda-Levin et al., 2005; Wojnicki et al., 2006; Cottone et al., 2008; Berner et al., 2009). In Chapter 4 I examined whether binge feeding of palatable food altered the ability of baclofen to modulate dopamine release in comparison to unlimited access to palatable food or standard chow.

As with all behavioural studies involving baclofen, sedative effects must be considered. When baclofen was given daily so that tolerance developed to the sedative effects of baclofen, baclofen increased food consumption, therefore it is possible that the sedative effect of an acute injection of a high concentration of baclofen masks baclofen induced hyperphagia (Bains and Ebenezzer, 2013). Together, these studies highlight the complex nature of the role of the GABA<sub>B</sub> receptor in feeding behaviour as well the importance of carefully controlled studies when examining drug effects on food consumption.

## **The mesolimbic dopamine system**

### **Role of NAc dopamine in behaviours**

The mesolimbic dopamine system has a major role in determining goal or reward directed behaviours and has long been implicated in drug seeking and addiction (De Wit and Wise, 1977; Yokel and Wise, 1978; Fumagalli et al., 1998). The mesolimbic dopamine system also modulates feeding behaviours, with an important role in regulating non-homeostatic feeding undertaken for reasons other achieving adequate nutrient and energy requirements (Bassareo and Di Chiara, 1999; Peciña et al., 2003; Berthoud, 2004).

Mesolimbic dopamine neurons originate in the VTA and project to the NAc (Swanson, 1982; Albanese and Minciacchi, 1983; Fallon, 1988). Dopamine release in the NAc encodes salience and incentive value and is important for shaping motivated behaviours (Phillips et al., 2003; Oleson et al., 2012; Sugam et al., 2012). The timing of dopamine release into the NAc closely follows the presentation/receipt of an unexpected reward and the concentration of dopamine

released in the NAc scales with the perceived reward value (Phillips et al., 2003; Roitman, 2004; Day et al., 2007; Gan et al., 2010).

As animals learn that environmental cues can predict reward dopamine release switches from appearing upon presentation of the reward to occurring upon the cues that predict the reward (Day et al., 2007). This helps guide behaviours to obtain the reward (Phillips et al., 2003; Oleson et al., 2012; Sugam et al., 2012; Saunders et al., 2013). This has important behavioural implications as there are many environmental cues predicting food reward (Berthoud, 2012). The concentration of dopamine released into the NAc also depends on motivated state, for example; if you are hungry cues predicting food will release more dopamine than if you are satiated (Bassareo and Di Chiara, 1999).

The NAc is divided into 2 sub-regions, the core and shell which differ anatomically and functionally. The NAc core is more important in reward seeking behaviours associated with reward predicting cues while the NAc shell is more important in reward seeking behaviours associated with reward predicting context (Bossert et al., 2007; Floresco et al., 2008; Chaudhri et al., 2010; Ambroggi et al., 2011). In addition the sub-regions are differentially altered by a sensitising cocaine regimen. The effect of cocaine on dopamine release in the NAc core is enhanced after a sensitising regimen of cocaine, while the effect of cocaine on dopamine release in the NAc shell is unchanged or reduced (Cadoni et al., 2000). For these reasons research undertaken in this thesis focused on the NAc core. Approximately 95% percent of neurons of the NAc are GABAergic projection neurons named medium spiny neurons (MSNs).

The remaining 5% of neurons include tonically active cholinergic interneurons and GABAergic interneurons (Adler et al., 2013).

### **Dopamine receptors**

There are 5 subtypes of dopamine receptors, which are classified as D1-like or D2-like. D1-like receptors include the D1 and D5 receptors while the D2-like receptors include the D2, D3 and D4 receptors (Missale et al., 1998). D1-like receptors are expressed on non-dopaminergic neurons. They are expressed abundantly throughout the NAc on MSNs that make up the direct pathway and couple to the stimulatory  $G\alpha_{s/olf}$  G-protein which activates adenylate cyclase increasing cAMP production (Spano et al., 1978; Gerfen and Surmeier, 2011). This can lead to increases in ionic currents through NMDA receptors, L-type VGCCs or  $Na^+$  channels (Missale et al., 1998; Surmeier et al., 2010).

D2-like receptors couple to the inhibitory  $G\alpha_{i/o}$  G-protein. Similar to  $GABA_B$  receptors, activation of D2 receptors inhibits adenylate cyclase, activates GIRK channels and inhibits VGCCs (Dal Toso et al., 1989; Monsma et al., 1989; Cardozo and Bean, 1995; Perra et al., 2011). D2 receptors are the major receptor subtype involved in feedback inhibition of dopamine release (Imperato and Di Chiara, 1988; Yokoo et al., 1988; Schmitz et al., 2002; Beckstead et al., 2004; Perra et al., 2011). In addition to sites on dopamine neuron terminals D2 receptors are expressed on cholinergic interneurons, glutamatergic terminals and on indirect pathway MSNs (Dawson et al., 1988; Bamford et al., 2004; Ding et al., 2010; Gerfen and Surmeier, 2011; Straub et al., 2014).

## **Regulation of extracellular dopamine concentration**

### **Dopamine neuron firing**

The firing pattern of dopamine neurons plays a major role in regulating the concentration of dopamine released from terminals (Chergui et al., 1994; Floresco et al., 2003; Venton et al., 2003; Zhang et al., 2009a). VTA dopamine neurons typically fire tonically at a low frequency; however, after salient stimuli such as unpredicted reward presentation/receipt, or cues that predict a reward, dopamine neurons discharge bursts of action potentials at high frequencies (Grace and Bunney, 1984a, 1984b; Zweifel et al., 2009). Burst firing of dopamine neurons increases the concentration of dopamine released from their terminations (Chergui et al., 1994; Floresco et al., 2003; Venton et al., 2003; Zhang et al., 2009a). As a consequence, the concentration of dopamine released at terminal regions is a function of VTA dopamine neuron cell excitability. Glutamatergic input at NMDA receptors on dopamine neurons has a critical role in the transition from tonic to burst firing (Overton and Clark, 1992; Chergui et al., 1993; Komendantov et al., 2004). Feedback inhibition of dopamine neuron excitability is achieved by somatodendritic release of dopamine acting at postsynaptic D2 autoreceptors coupled to GIRK channels (Beckstead et al., 2004; Ford et al., 2007). GABA acting at GABA<sub>A</sub> receptors is important for regulating the pattern of burst firing (Komendantov et al., 2004) while activation of GABA<sub>B</sub> receptors on somatodendrites inhibits dopamine neuron excitability and burst firing (Erhardt et al., 2002).

### **Dopamine clearance**

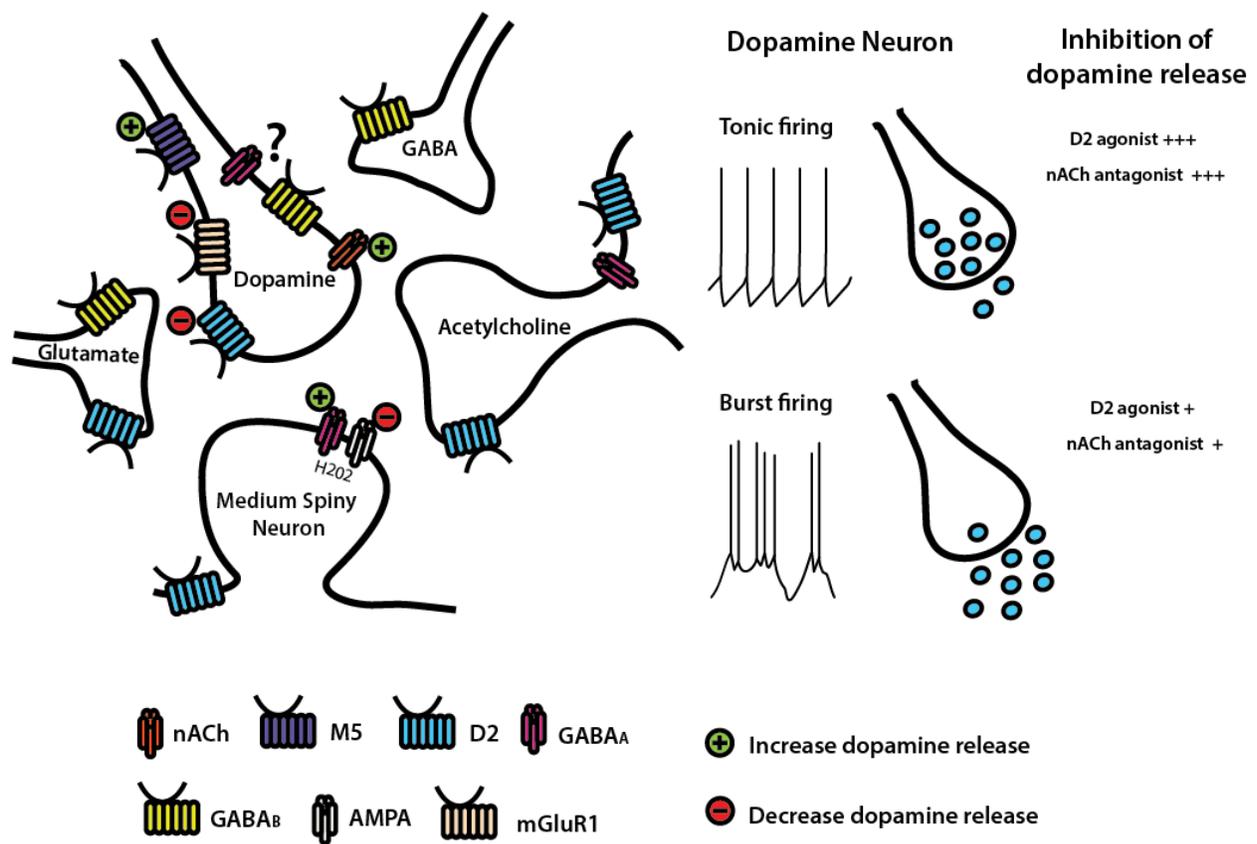
The concentration of extracellular dopamine is a balance between release and clearance, with clearance being a function of diffusion and reuptake (Wightman and Zimmerman, 1990).

Dopamine reuptake by dopamine transporters is an important mechanism of clearance in the NAc (Nirenberg et al., 1997a, 1997b). Dopamine transporters are localised perisynaptically (Nirenberg et al., 1996, 1997b). They bind dopamine and, along with 2 Na<sup>+</sup> ions and one Cl<sup>-</sup> ion, transport dopamine from the extracellular space into the cytosol of the dopaminergic nerve terminal (Gu et al., 1994). Cocaine increases extracellular dopamine by inhibiting dopamine transporters (Ritz et al., 1987). Amphetamine or methamphetamine act as substrates for the dopamine transporter (Sulzer et al., 1995; Jones et al., 1998b). They are transported into dopaminergic nerve terminal where they promote release of dopamine from synaptic vesicles into the cytoplasm. Consequently the activity of the dopamine transporter is reversed, with the transporter mediating the transport of dopamine from the cytosol into the extracellular space (Sulzer et al., 1995; Jones et al., 1998b).

### **Terminal modulation of dopamine release**

A variety of neurotransmitters can modulate dopamine release via actions at dopaminergic nerve terminals in the NAc. The release of dopamine from dopaminergic nerve terminals has been shown to be dependent upon Ca<sup>2+</sup> (Phillips and Stamford, 2000; Cragg, 2003; Chen et al., 2006, 2011; Ford et al., 2010). Inhibition of release can be achieved by a direct inhibition of Ca<sup>2+</sup> channels or by increasing axonal conductance for K<sup>+</sup> which shunts axon potential incursion into the nerve terminal. Below are summarised some of the ways that neurotransmitters can regulate the concentration of dopamine released from dopaminergic nerve terminals, focusing on studies carried out using fast scan cyclic voltammetry in brain slices, which is the technique used for the research undertaken in Chapters 3 and 4. This technique enables determination of direct effects on dopaminergic nerve terminals by using stimulations that do not evoke GABA or

glutamate release in sufficient concentrations to alter dopamine release (reviewed by Zhang and Sulzer, 2012). As well the frequency of applied electrical stimulations can be altered to mimic dopamine release under both tonic and burst firing of dopamine neurons (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Ferris et al., 2013). Figure 1.2 demonstrates the distribution of receptors in the NAc and their effect on dopamine release elicited by tonic or burst firing of dopamine neurons.



**Figure 1.2 Receptors modulating dopamine release.**

Simplified schematic diagram showing the distribution of receptors in the NAc and their effect on dopamine release as measured by fast scan cyclic voltammetry in brain slices. Schematic also demonstrates increased dopamine release induced by burst firing of dopamine neurons compared to tonic firing of dopamine neurons.

## **ACh**

The major source of ACh in the NAc is from tonically active cholinergic interneurons. Activation of the Ca<sup>2+</sup> permeable nicotinic ACh (nACh) receptors expressed on dopaminergic terminals substantially increases dopamine release (Zhou et al., 2001; Zoli et al., 2002; Salminen et al., 2004; Zhang and Sulzer, 2004). Preventing the action of ACh at nACh receptors, either by inhibiting ACh release or blocking nACh receptors, decreases dopamine release elicited by stimulations mimicking tonic firing of dopamine neurons, but has minimal effect on dopamine release elicited with stimulations mimicking burst firing (Rice and Cragg, 2004; Zhang and Sulzer, 2004). Consequently, inhibition of ACh action at nACh receptors amplifies the difference between dopamine release in response to salient stimuli compared with dopamine release under tonic firing conditions, referred to as the “signal to noise ratio” of dopamine release (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Zhang et al., 2009b). G-protein coupled muscarinic ACh receptors can also modulate dopamine release, for example, M5 receptors on dopamine neuron nerve terminals can facilitate dopamine release (Zhang et al., 2002; Threlfell et al., 2010).

## **Dopamine**

Dopamine acting at D2 autoreceptors produces strong feedback inhibition of dopamine release (Schmitz et al., 2002). D2 receptors can negatively couple to N- and P/Q type VGCCs (Cardozo and Bean, 1995); however, it is unknown if this is the mechanism responsible for dopamine mediated inhibition of terminal dopamine release. Evidence for the contrary includes

investigation of D2 action on glutamatergic autapses of cultured midbrain neurons which showed no effect on calcium influx (Congar et al., 2002). Instead D2 receptor activation may inhibit dopamine release probability by activating voltage-gated K<sup>+</sup> channels (Congar et al., 2002; Fulton et al., 2011; Martel et al., 2011). Similar to the effect of antagonising nACh receptors, the ability of D2 receptors to inhibit dopamine release is reduced when dopamine release is evoked with electrical stimulations that mimic burst firing of dopamine neurons (Zhang and Sulzer, 2004; Zhang et al., 2009a).

D2 receptor activation also reduces the concentration of dopamine released by other mechanisms over longer time scales such as decreasing the activity of tyrosine hydroxylase (rate-limiting enzyme in catecholamine synthesis) by a cAMP dependent mechanism (Onali and Olanas, 1989; Salah et al., 1989; Lindgren et al., 2001). Activation of D2 receptors on dopamine terminals can also alter dopamine reuptake (Meiergerd et al., 1993; Cass and Gerhardt, 1994; Dickinson et al., 1999; Mayfield and Zahniser, 2001; Bolan et al., 2007).

### **Glutamate**

Ionotropic receptors for glutamate (NMDA, AMPA and kainic acid receptors) are not expressed on dopamine neuron terminals (Bernard and Bolam, 1998), but have been shown to regulate dopamine release indirectly. Glutamate and GABA can be released in sufficient quantities to modulate dopamine release using a pulse train electrical stimulus (30 pulses, 10 Hz) (Avshalumov and Rice, 2003; Avshalumov et al., 2003). Activation of AMPA receptors on MSNs by electrically released glutamate initiates a signalling cascade that results in retrograde diffusion of H<sub>2</sub>O<sub>2</sub> across the synapse (Avshalumov and Rice, 2003; Avshalumov et al., 2003). H<sub>2</sub>O<sub>2</sub>

inhibits dopamine release by activating  $K_{ATP}$  channels on dopaminergic nerve terminals (Avshalumov and Rice, 2003; Avshalumov et al., 2003). In Chapters 3 and 4 I evoked dopamine release with electrical stimuli that did not result in sufficient release of GABA or glutamate to modulate dopamine release in order to focus on direct actions on dopaminergic nerve terminals (Zhang and Sulzer, 2012).

mGluRs can also modulate terminal dopamine release. Group 1 mGluR receptors, which couple to the  $G_q$  G-protein are expressed on dopamine terminals (Paquet and Smith, 2003). Activation of group 1 mGluR receptors inhibits terminal dopamine release by mobilisation of internal  $Ca^{2+}$  stores. This results in activation of  $Ca^{2+}$  activated  $K^+$  channels (Zhang and Sulzer, 2003).

## **GABA**

In experiments using pulse train electrical stimulations  $GABA_A$  receptor activation enhanced dopamine release by inhibiting  $H_2O_2$  production in MSNs (Avshalumov and Rice, 2003).  $GABA_A$  receptors are present on cholinergic interneurons (Straub et al., 2014) and may also be present on dopaminergic nerve terminals. Unpublished results mentioned in a review by Zhang & Sulzer suggest that a  $GABA_A$  agonist can inhibit dopamine release elicited by a single pulse stimulus (Zhang and Sulzer, 2012). Thus, the actions of GABA agonists on dopamine release need further study.

## **$GABA_B$ modulation of dopamine release**

$GABA_B$  receptors are expressed on both dopamine neurons and GABA neurons of the VTA. On GABA neurons they are found post-synaptically where they couple to GIRK channels, and pre-synaptically where they inhibit  $Ca^{2+}$  dependent GABA release (Cruz et al., 2004; Labouèbe et al.,

2007). The postsynaptic GABA<sub>B</sub> receptors on dopamine neurons also couple to GIRK channels and are located at sites where they are activated by GABA release from neurons originating outside the VTA (Sugita et al., 1992; Cruz et al., 2004; Labouèbe et al., 2007).

Baclofen has no effect on the membrane properties of MSNs suggesting that GABA<sub>B</sub> receptors are not expressed on the principal cell type of the NAc (Uchimura and North, 1991; Molnár et al., 2009), however, this does not exclude the possibility of GABA<sub>B</sub> receptors that can initiate signalling cascades that do not affect the membrane properties of neurons (Charara et al., 2000; Lacey et al., 2005). GABA<sub>B</sub> receptors are expressed on GABAergic and glutamatergic terminals in the NAc and patch clamp studies have shown that baclofen inhibits both inhibitory and excitatory mini post-synaptic currents onto MSNs (Uchimura and North, 1991; Lacey et al., 2005; Molnár et al., 2009). Ultrastructural studies in monkeys suggest that GABA<sub>B</sub> receptors are also located on dopamine neuron terminals (Charara et al., 2000).

Intraperitoneal injection of baclofen decreases dopamine release in the NAc as measured by microdialysis (Fadda et al., 2003; Fu et al., 2012). This could be due to actions in the VTA, in the NAc or both. Studies have reported that intra VTA application of baclofen decreases both somatodendritic dopamine release and release of dopamine in the NAc (Klitenick et al., 1992; Yoshida et al., 1994; Westerink et al., 1996; Xi and Stein, 1998, 1999). In vivo microdialysis studies have also demonstrated that baclofen can inhibit dopamine release when applied into the NAc (Xi et al., 2003). This could be due to effects at dopaminergic, glutamatergic or GABAergic terminals. Because little is known about how the GABA<sub>B</sub> receptor modulates dopamine release in the NAc, I address this question in Chapter 3.

## **Cocaine induced alterations in GABA<sub>B</sub> receptor expression and signalling**

Cocaine alters GABA<sub>B</sub> receptor signalling in the VTA, NAc and the medial prefrontal cortex (mPFC). Baclofen-evoked currents in VTA neurons were reduced by exposure to cocaine (Arora et al., 2011; Padgett et al., 2012). A single injection of cocaine decreased the slow inhibitory post synaptic current and baclofen-induced K<sup>+</sup> current in GABA neurons of the VTA (Padgett et al., 2012). Reports on whether GABA<sub>B</sub> receptors on VTA dopamine neurons are similarly altered are conflicting. Padgett et al. (2012) reported that baclofen-evoked K<sup>+</sup> currents in dopamine neurons were unaltered by a single injection of cocaine while Arora et al. (2011) reported that they were. The reason for the difference in findings is unclear as both groups used similar experimental procedures, including the dose of cocaine (15 mg/kg i.p.) and breed and age ranges of mice (C57BL/6J, p28-42 or p15-35). Estrogen can differentially affect cocaine-induced changes in baclofen stimulated GTP-γ-S binding in the VTA (Febo and Segarra, 2004) which could account for the discrepancies, although, both studies used male and female mice.

Inhibitory effects on neurotransmitter release mediated by baclofen applied to the mPFC are reduced in cocaine sensitised rats (Jayaram and Steketee, 2004). 5 daily injections of cocaine (but not a single injection) caused an approximately 50% reduction in postsynaptic baclofen-evoked K<sup>+</sup> currents in pyramidal neurons of layer 5 and 6 in the mPFC, recorded 24 hours after the last injection (Hearing et al., 2013). This was correlated with a depolarised resting membrane potential and enhanced excitability of these neurons. The effect on the baclofen-evoked K<sup>+</sup> currents persisted for up to 6 weeks after the last injection. The reduction in baclofen mediated K<sup>+</sup> currents was dependent upon D1-like, but not D2-like, receptor activation. No differences in Kir3.2 or GABA<sub>B1</sub> mRNA or total protein levels were observed, but there was a

significant decrease in the membrane expression of Kir3.2 and GABA<sub>B1</sub> protein, concomitant with decreased serine phosphorylation of the GABA<sub>B2</sub> subunit (Hearing et al., 2013).

Cocaine has also been shown to alter GABA<sub>B</sub> receptor signalling in the NAc. Baclofen-stimulated GTP-γ-S binding in the NAc was reduced after 3 weeks withdrawal from chronic cocaine administration (Xi et al., 2003). This decrease was associated with an increase in extracellular GABA levels in the NAc and decreased serine phosphorylation of the GABA<sub>B2</sub> subunit, indicating decreased stability of the GABA<sub>B</sub> receptor at the cell membrane. Other studies have also reported an increase in extracellular GABA in the NAc after chronic cocaine administration (Wydra et al., 2013). It has been hypothesised that increased tone at GABA<sub>B</sub> receptors could lead to agonist induced desensitisation (Xi et al., 2003; Frankowska et al., 2008). Interestingly, while in vivo microdialysis studies revealed that baclofen modulation of GABA release was altered after withdrawal from chronic cocaine treatment there were no changes in the ability of baclofen to modulate dopamine release (Xi et al., 2003).

A reduction in radiolabelled GABA<sub>B</sub> receptor antagonist ([<sup>3</sup>H]CGP 54626) binding in the NAc after 10 days withdrawal from chronic cocaine was demonstrated by Frankowska et al. (2008) (Frankowska et al., 2008). A decrease was also observed immediately after ending cocaine treatment, indicating that withdrawal was not necessary for alterations in GABA<sub>B</sub> receptor binding (Frankowska et al., 2008). Importantly it was demonstrated that the decrease in the NAc occurred whether cocaine was self-administered or administered by the experimenter in a “yoked” procedure (average of 171 – 178 mg/kg per day). In contrast with these studies an earlier study found no differences in the EC<sub>50</sub> or potency of baclofen to activate GABA<sub>B</sub>

receptors in the NAc immediately after ending chronic cocaine administration (15mg/kg, 3x per day, i.p.) (Kushner and Unterwald, 2001).

These studies indicate that GABA<sub>B</sub> receptor function in the NAc may be altered after chronic cocaine administration. However, it has not previously been investigated whether all GABA<sub>B</sub> receptors in the NAc are equally affected. Therefore, in Chapter 3 I used fast scan cyclic voltammetry in brain slices to determine whether the function of GABA<sub>B</sub> receptors located on dopaminergic nerve terminals in the NAc was altered by sensitising cocaine administration.

### **Diet induced changes in the mesolimbic dopamine system**

Extended exposure to a high fat diet or diet induced obesity has been proposed to induce hypofunction of the neural systems mediating reward (Johnson and Kenny, 2010). For example, the dopamine response to palatable foods is blunted in obese humans (Stice et al., 2008a, 2008b; Green et al., 2011) and studies in rodents have shown decreased extracellular dopamine in the NAc after extended access to a high fat or cafeteria diet (Geiger et al., 2009). Obese rodents show less motivation for obtaining food rewards (Davis et al., 2008; Shin et al., 2011a) and have elevated reward thresholds (Johnson and Kenny, 2010). In addition, some of the effects of drugs of abuse are decreased in obese rodents compared to chow-fed controls. For example, conditioned place preference for amphetamine is reduced in rats with 12 weeks access to a high fat diet (Davis et al., 2008) and dopamine release evoked by amphetamine is reduced after a high fat or cafeteria diet (Geiger et al., 2009; Speed et al., 2011). The extent of obesity may not be important. Obesity prone rats had increased motivation for food and

increased methamphetamine evoked dopamine release compared with obesity resistant rats after both groups had 8 weeks access to a high fat diet (Narayanaswami et al., 2013).

Studies in both humans and rodents have demonstrated both decreases (Hamdi et al., 1992; Colantuoni et al., 2001; Wang et al., 2001; Hajnal et al., 2008; Johnson and Kenny, 2010; Narayanaswami et al., 2013) and increases (Huang et al., 2005; South and Huang, 2008; Sharma and Fulton, 2013) in D2 dopamine receptor expression in the dorsal/ventral striatum after extended access to high fat or cafeteria style diets. Putative reasons for differences include the availability of choice (van de Giessen et al., 2012) or the macronutrient composition of the diet (van de Giessen et al., 2013). A decrease in the expression and/or function of striatal dopamine transporters after extended access to a high fat or cafeteria diet has also consistently been reported (South and Huang, 2008; Speed et al., 2011; Cone et al., 2013; Narayanaswami et al., 2013).

The effects of restricted, intermittent food access that results in binge-like feeding may contrast to that of extended, un-restricted access to a highly palatable diet. Binge eating in rats has been shown to increase motivation to obtain cocaine (Puhl et al., 2011) and sucrose bingeing enhances the locomotor effect of cocaine (Gosnell, 2005) and amphetamine (Avena and Hoebel, 2003). In addition, binge eating disorders have a high co-morbidity with substance use disorders in humans even though obese humans are less likely to abuse drugs (Warren et al., 2005; Conason et al., 2006; Simon et al., 2006).

While studies have reported changes in D2 receptor expression after diet induced obesity, it has not been determined whether the ability of D2 receptor activation to inhibit dopamine

release is affected. In addition, no one has studied whether GABA<sub>B</sub> receptor function or expression is altered by diet induced obesity or binge feeding. In Chapter 4 I examined whether inhibition of dopamine release mediated by D2 or GABA<sub>B</sub> agonists was altered by unlimited or restricted access to a palatable cafeteria diet.

## **Chapter 2 Isovaline does not activate GABA<sub>B</sub> receptor-coupled K<sup>+</sup> currents in either GABA<sub>B</sub> expressing AtT-20 cells or in cultured rat hippocampal neurons**

### **Introduction**

Receptors for GABA, especially of the GABA<sub>B</sub> type, are a promising target in analgesia. However, the use of the prototypical GABA<sub>B</sub> agonist, baclofen, is limited due to the development of severe side effects (Enna and McCarson, 2006). Isovaline is an unusual non-proteinogenic amino acid that is anti-nociceptive without side effects typical of GABA<sub>B</sub> agonists (MacLeod et al., 2010; Whitehead et al., 2012). A component of the analgesia produced by isovaline is attributable to activation of GABA<sub>B</sub> receptors (Whitehead et al., 2012).

GABA<sub>B</sub> receptors are obligate heterodimers, made up of a GABA<sub>B1</sub> and a GABA<sub>B2</sub> subunit (Jones et al., 1998a; Kaupmann et al., 1998; White et al., 1998). Surprisingly, there are no pharmacologically distinct GABA<sub>B</sub> receptor subtypes (Bowery et al., 2002; Bettler and Tiao, 2006). Critical residues for orthosteric agonist and antagonist binding are located on the GABA<sub>B1</sub> subunit VFT (Galvez et al., 1999, 2000a; Geng et al., 2013), while allosteric modulators of the GABA<sub>B</sub> receptor act at sites on the GABA<sub>B2</sub> subunit (Binet et al., 2004). GABA<sub>B</sub> receptors are GPCRs that associate with the pertussis toxin sensitive G<sub>αi/o</sub> family of G-proteins (Franek et al., 1999). Conventional cellular effects of GABA<sub>B</sub> receptor agonism include inhibition of adenylate cyclase, G<sub>βγ</sub>-mediated activation of GIRK channels and G<sub>βγ</sub>-mediated inhibition of VGCCs (Xu and Wojcik, 1986; Bowery et al., 2002; Padgett and Slesinger, 2010).

Isovaline's action at GABA<sub>B</sub> receptors is atypical as demonstrated in thalamic neurons of brain slices. Compared to R-baclofen, R-isovaline-evoked K<sup>+</sup> currents are slow in onset and long

lasting (Cooke et al., 2009, 2012). Also in contrast to R-baclofen, the response to R-isovaline is blocked by pre-application of a GABA<sub>B</sub> receptor antagonist, but not by application of the GABA<sub>B</sub> antagonist subsequent to the initiation of the K<sup>+</sup> current (Cooke et al., 2012). Furthermore, some neurons that respond to R-baclofen do not respond to R-isovaline (Cooke et al., 2012). The ability of R-isovaline to activate currents in sensitive neurons does not appear to result from GABA release and subsequent postsynaptic activation of GABA<sub>B</sub>-mediated K<sup>+</sup> currents, since inhibition of other GABA-mediated currents does not alter R-isovaline responses (Cooke, 2010). Here I test the effects of R-isovaline on GABA<sub>B</sub> receptors in a neuronal expression system as well as in isolated hippocampal neurons natively expressing GABA<sub>B</sub> receptors.

## **Materials and methods**

### **AtT-20 cell culturing, transfection and electrophysiology**

Mouse pituitary AtT-20 cells (Furth et al., 1953; Buonassisi et al., 1962) obtained from Dr. C. Chavkin's laboratory at the University of Washington (March, 2010) were grown in Gibco high glucose Dulbecco's modified Eagle medium (Invitrogen) with 1% fetal bovine Serum, 10% horse serum, penicillin-streptomycin and 0.2 mM L-glutamine and kept in an incubator at 37°C with 5% CO<sub>2</sub> in air. Cells were passaged every 2-3 days. For transient transfection cells were plated in a 35mm culture dish at 90% confluency and left for 24 hours. Cells were then co-transfected overnight in serum containing media using Lipofectamine 2000 (Invitrogen, Life Technologies, Burlington, ON) following the manufacturer's protocols. Cells were co-transfected with cDNA for the GABA<sub>B1a</sub> and GABA<sub>B2</sub> subunits as well as green fluorescent protein (GFP) to aid identification of transfected cells (1:2:5 ratio of GFP:GABA<sub>B1a</sub>:GABA<sub>B2</sub>). The following day the

transfection media were removed and cells were re-plated onto poly-D-lysine coated glass cover slips which were then incubated for a further 24 hours before use.

To confirm membrane expression of the GABA<sub>B</sub> receptor in cells that were suitable for patching (isolated from one another), I performed immunohistochemistry for the GABA<sub>B1</sub> subunit which is unable to traffic to the membrane without associating with a GABA<sub>B2</sub> subunit (Jones et al., 1998a; Kaupmann et al., 1998; White et al., 1998). Cells were fixed with 4% formaldehyde for 30 minutes then blocked with 0.5% bovine serum albumin for 1 hour. Cells were incubated with or without mouse anti-GABA<sub>B1</sub> (1:1000 dilution, ab55051, Abcam, Toronto) overnight at 4°C. Goat anti-mouse conjugated to Alexa Fluor 546 (Life Technologies) was applied at a 1:200 dilution for 1 hour at room temperature. DAPI (4',6-diamidino-2-phenylindole) was applied at 1:10,000 dilution for 5 minutes to stain the nuclei. Coverslips were washed with phosphate buffered saline 3 times between each step. The glass coverslips were mounted onto glass slides and left to dry for at least 72 hours. Confocal microscopy was performed using an Olympus FluoView FV1000 confocal microscope with a 60x objective (Tokyo, Japan).

For electrophysiological recordings, cells were removed from the incubator and the medium was replaced at room temperature with high K<sup>+</sup> extracellular solution containing (in mM): NaCl (130), KCl (20), CaCl<sub>2</sub> (2), MgCl<sub>2</sub> (1), HEPES (10), glucose (35), and 0.1-1 μM tetrodotoxin. The solution had a pH = 7.4 (adjusted with NaOH) and an osmolality of 330 ± 5 mOsm/kg. The glass coverslip was cut into sections and placed into a 50 μL fast exchange diamond bath (Warner Instruments, Hamden, CT) constantly perfused (~2 ml/min) with extracellular solution. Recording electrodes were made out of thin-walled borosilicate glass (World Precision

Instruments, Sarasota, FL) and had a resistance between 3-5 M $\Omega$  when filled with an intracellular pipette solution containing (in mM): K-gluconate (130), NaCl (20), MgCl<sub>2</sub> (1), EGTA (10), glucose (10), HEPES (10), MgATP (5) and Na<sub>3</sub>GTP (0.1) with pH = 7.2 (NaOH/HCl) and an osmolality of 310  $\pm$  3 mOsm/kg. The calculated Nernst equilibrium potential for K<sup>+</sup> was approximately -47 mV at 20°C. Cells were visualized with an Axiovert 25 inverted fluorescent microscope (Zeiss, Germany). Whole cell recordings from fluorescing cells were performed using a List EPC 7 amplifier (HEKA, Germany). Recordings were filtered at 3 kHz, digitized at 10 kHz and analysed using pClamp 8.2 software (Molecular Devices, Sunnyvale, CA). Cells were voltage clamped with a holding potential was -50 mV. The current-voltage (IV) relationship was measured each minute by applying a family of 400 ms voltage steps, from -100 mV to +20 mV, in 10 mV increments. A junction potential of -12.5 mV was accounted for offline. The current was measured as the average current in the last 100 ms of each voltage step. In all experiments except one, drugs were applied via the bath for 2- 5 min, and then washed off. In one experiment R-isovaline was added into the intracellular pipette solution. Cells were held in voltage clamp for an extra 5 min before further drug application to allow for intracellular dialysis. In several cells GABA<sub>B</sub> action was confirmed by blockade with a GABA<sub>B</sub> antagonist.

### **Hippocampal cell culturing and electrophysiology**

Cultured hippocampal neurons were prepared from 18 day old Sprague-Dawley rat embryos (male and female) following methods described in detail by Xie et al. (Xie et al., 2000). Rat housing and culture preparation methods were in accordance with the Canadian Council on Animal Care and approved by the University of British Columbia Animal Care Committee. Pregnant rats were sacrificed with 5% CO<sub>2</sub> and care was taken to ensure an absence of

nociceptive response prior to decapitation and removal of embryos. Neurons were plated on poly-L-lysine coated glass coverslips at a density of 130 cells/mm<sup>2</sup> and were incubated with 5% CO<sub>2</sub> in air at 37°C for 14 to 28 days until used for electrophysiological analysis *in vitro*.

On the day of recording cultured hippocampal neurons were removed from the incubator and the glass coverslip was placed into a culture dish filled with room temperature (~22°C) extracellular solution containing (in mM): NaCl (140), KCl (5.4), CaCl<sub>2</sub> (2), MgCl<sub>2</sub> (1), HEPES (20) and glucose (20) at pH = 7.4 and 325 ± 5 mOsm/kg. In some experiments, hippocampal neurons were incubated with R-isovaline (50 µM) in extracellular solution for at least an hour before recordings. Recording electrodes (4-5.5 MΩ) were filled with a solution containing (in mM): K-gluconate (120), KCl (20), NaCl (10), HEPES (10), EGTA (5) MgATP (3) and Na<sub>2</sub>GTP (0.2) at pH 7.2 and 305 ± 5 mOsm/kg. Cells were voltage clamped at -80 mV and the membrane current was constantly recorded. To enhance inward K<sup>+</sup> currents, a high [K<sup>+</sup>] extracellular solution, whereby 20 mM NaCl was replaced with equimolar KCl, was used to examine drug effects. Drugs were mixed with the high [K<sup>+</sup>] extracellular solution and applied via the bath perfusate for a minimum of 30 s and a maximum of 5 min. The average current during 20 s of the peak drug effect was measured and compared with the current in the last 20 s before the drug was washed in (baseline). In the case of no obvious drug effect, the average current within the last 20 s before drug wash-off was measured. Recordings were filtered at 3 kHz, digitized at 10 kHz and analysed using pClamp 8.2 software.

## Drugs

Extracellular solution was prepared on the day of the experiment. Intracellular solution was prepared and frozen in aliquots and used within a week. Stock solutions of baclofen HCl (R-enantiomer, Sigma-Aldrich, St. Louis, MO), GABA (Sigma-Aldrich), CGP 52432 (Tocris, UK) and R-isovaline HCl or S-isovaline HCl (BioFine International, Vancouver, BC), were made using double distilled H<sub>2</sub>O and kept at 4°C. Final drug concentrations were prepared on the day of experiment.

## Statistical analysis

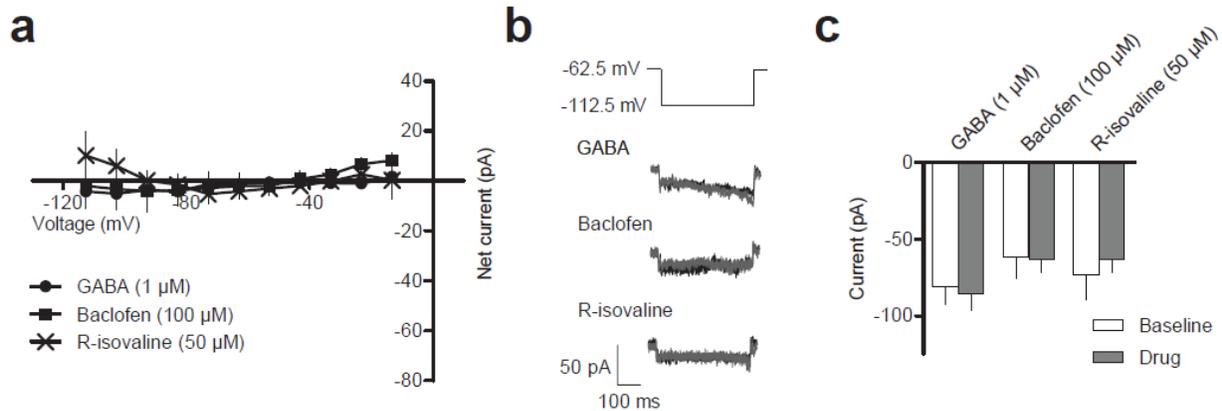
For data from AtT-20 cells, agonist action at the GABA<sub>B</sub> receptor was defined as the ability to induce a current that was inwardly rectifying and reversed at approximately E<sub>K</sub>. The net current was determined by subtracting the baseline current from the current during drug application at each voltage step. The IV relationships were then plotted to determine whether the net current was inwardly rectifying. To determine the reversal potential, the linear section of the baseline subtracted IV curve (-112.5 mV to -52.5 mV) for each recording was fitted using linear regression, the point at which Y=0 for each curve was determined and mean ± SEM and 95% confidence interval calculated. For clarity, example currents from AtT-20 cells depict only the current recorded at the maximum hyperpolarising step (-112.5 mV). Bar graphs display the untransformed current at the maximum hyperpolarising step (-112.5 mV) for AtT-20 cells or at the holding potential (-80 mV) for hippocampal cells. For analysis of the effect of intracellular R-isovaline, the baseline current was measured by stepping to -112.5 mV from a holding potential of -62.5 mV at t = 1 min. This baseline value was then subtracted from all subsequent measurements. Repeated measures 1 or 2-way analysis of variance (ANOVAs) with Bonferroni's

post-hoc test were used as appropriate to test for drug induced changes in currents. For analysis of changes in the R-baclofen-evoked current in hippocampal cells, the baclofen-evoked current (net current) was determined by subtracting the baseline current from the current during application of R-baclofen. A 1-way ANOVA was used to compare the magnitude of R-baclofen-evoked currents. Data are expressed as mean  $\pm$  SEM. "n" refers to the number of cells. Results were considered significant if  $p < 0.05$ .

## Results

### **GABA<sub>B</sub> receptors express and are functional in transfected AtT-20 cells**

I examined whether untransfected AtT-20 cells exhibited plasmalemmal responses to applications of GABA<sub>B</sub> agonists or R-isovaline by determining their effects at various membrane voltages. Figure 2.1 a-c shows that there were no statistically significant effects of GABA, baclofen or R-isovaline on the IV-relationships or on the maximal currents evoked with a hyperpolarising step to -112.5 mV (main drug effect  $F_{(1,10)} = 0.07$ ,  $p = 0.8$ ; repeated measures 2-way ANOVA). Bonferroni's post hoc test indicated no changes in the current after bath application of GABA (baseline:  $-80.9 \pm 11.5$  pA vs. GABA:  $-85.2 \pm 10.9$  pA, 1  $\mu$ M, 2 min,  $n = 4$ ), baclofen (baseline:  $-61.2 \pm 14.2$  pA vs. baclofen:  $-63.2 \pm 8.2$  pA, 100  $\mu$ M, 2 min,  $n = 4$ ) or R-isovaline (baseline:  $-73.3 \pm 16.3$  pA vs. R-isovaline:  $-63.2 \pm 8.6$  pA, 50  $\mu$ M, 5 min,  $n = 5$ ). These data, summarized in Figure 2.1 c, suggest that untransfected AtT-20 cells do not have GABA<sub>B</sub> receptors which couple to K<sup>+</sup> currents.

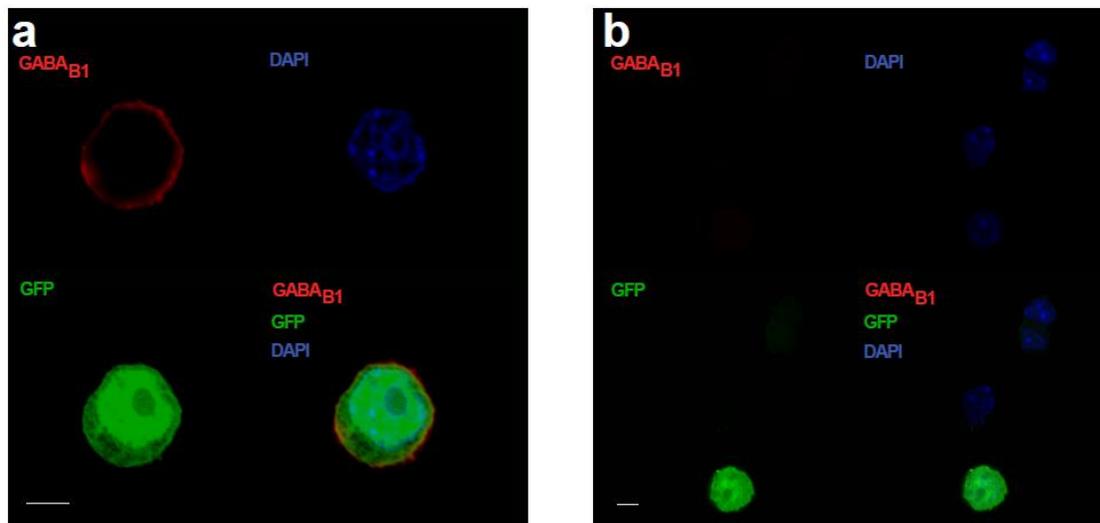


**Figure 2.1 GABA, baclofen and R-isovaline do not evoke currents in untransfected AtT-20 cells.**

a) Baseline subtracted IV curve for GABA (n = 4), baclofen (n = 4) and R-isovaline (n = 5). b) Example currents at the maximum hyperpolarising step (-112.5 mV, voltage step depicted above currents) at baseline (black) and on application (grey) of GABA, baclofen or R-isovaline. c) Summary graph showing currents recorded at -112.5 mV at baseline (open bars) and on application of GABA, baclofen, or R-isovaline (shaded bars). Data are represented as mean  $\pm$  SEM.

I then transiently transfected AtT-20 cells with the GABA<sub>B1a</sub> and GABA<sub>B2</sub> subunits and GFP. To confirm membrane expression of GABA<sub>B</sub> receptors in AtT-20 cells, I used a mouse monoclonal antibody to the GABA<sub>B1</sub> subunit, which is unable to traffic to the cell surface without GABA<sub>B2</sub> (Jones et al., 1998a; Kaupmann et al., 1998; White et al., 1998). Figure 2.2 a,b illustrates cell surface expression of GABA<sub>B1</sub> and colocalisation with GFP alongside the negative control. Next, I assessed if AtT-20 cells transiently transfected with both the GABA<sub>B1a</sub> and GABA<sub>B2</sub> subunits responded to GABA, baclofen or R- and S-isovaline. GABA (1 μM) applied for 2 min produced an inwardly rectifying current that was antagonised by co-application with 1 μM CGP 52432 (Figure 2.3 a). GABA evoked a maximum current of  $-41.0 \pm 9.7$  pA. Co-application of GABA and a GABA<sub>B</sub> antagonist, CGP 52432, reduced the current to  $-8.0 \pm 4.1$  pA (paired t-test, t = 4.316, df =

6,  $p = 0.005$ ,  $n = 7$ ). The reversal potential of the GABA evoked current was  $-52 \pm 3$  mV (95% CI:  $-59$  mV to  $-45$  mV,  $n = 7$ ).



**Figure 2.2** The  $GABA_{B1}$  subunit expresses at the membrane in transfected AtT-20 cells.

AtT-20 cells were transfected with both subunits for the  $GABA_B$  receptor along with GFP (shown in green). Immunohistochemistry was performed for the  $GABA_{B1}$  subunit (red) and cell nuclei were stained with DAPI (blue). a) Magnified image of an isolated cell demonstrating  $GABA_{B1}$  subunit protein at the cell membrane in a GFP positive cell. b) Negative control (no primary antibody for  $GABA_{B1}$ ). A merged image is shown at the bottom right of each square. Scale bars are 5  $\mu$ m.

As shown in Figure 2.3 b, baclofen (5  $\mu$ M, 2 min) also induced an inwardly rectifying current.

The maximum evoked current was  $-44.5 \pm 16.5$  pA. The mean reversal potential of the baclofen-evoked current was  $-44.0 \pm 2.0$  mV (95% CI:  $-48$  to  $-40$  mV,  $n = 6$ ). These data, summarised in

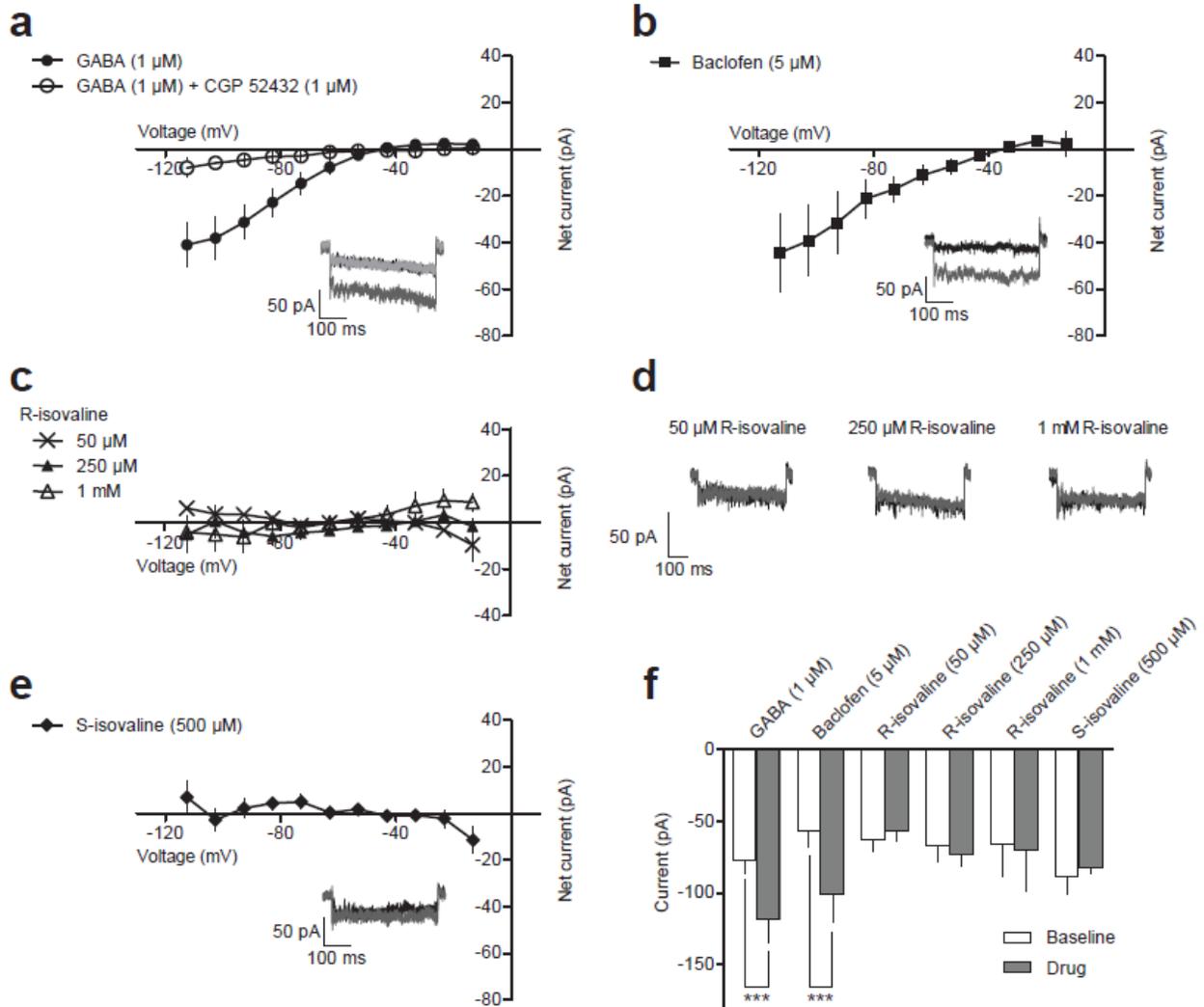
Figure 2.3 f, demonstrate that GABA and baclofen produce significant changes in current as determined by repeated measures 2-way ANOVA with Bonferroni's post hoc test (interaction

$F_{(5,29)} = 5.48$ ,  $p = 0.0011$ ; GABA  $-118.5 \pm 12.9$  pA vs baseline  $-77.4 \pm 8.8$  pA,  $p < 0.001$ ,  $n = 7$ ;

baclofen  $-101.4 \pm 12.3$  pA vs. baseline  $-56.9 \pm 10.9$  pA,  $p < 0.001$ ,  $n = 6$ )

### **R-isovaline did not activate GABA<sub>B</sub> receptor coupled K<sup>+</sup> currents**

In contrast to GABA and baclofen, R-isovaline (5 min) did not produce a statistically significant change in the measured current (Figure 2.3 c,d,f). R-isovaline currents at 50  $\mu$ M, a submaximal effective concentration in thalamocortical slices (Cooke et al., 2009), were  $-56.4 \pm 7.8$  pA vs. a baseline of  $-62.5 \pm 8.9$  pA ( $p > 0.05$ ,  $n = 6$ ). Furthermore, the mean current after application of 250  $\mu$ M R-isovaline, a ceiling concentration for imparting an effect on membrane conductance in thalamocortical slices (Cooke et al., 2009), was  $-72.8 \pm 8.3$  pA vs a baseline of  $-67.5 \pm 11.4$  pA ( $p > 0.05$ ,  $n = 8$ ) and at 1 mM R-isovaline, they were  $-70.3 \pm 28.8$  pA vs. a baseline of  $-66.1 \pm 23.0$  pA ( $p > 0.05$ ,  $n = 4$ ). A high concentration of the S-enantiomer of isovaline (500  $\mu$ M for 5 min, Figure 2.3 e,f) also did not produce a change from baseline current ( $-82.2 \pm 4.8$  pA vs baseline  $-89.2 \pm 11.3$  pA,  $p > 0.05$ ,  $n = 4$ ).

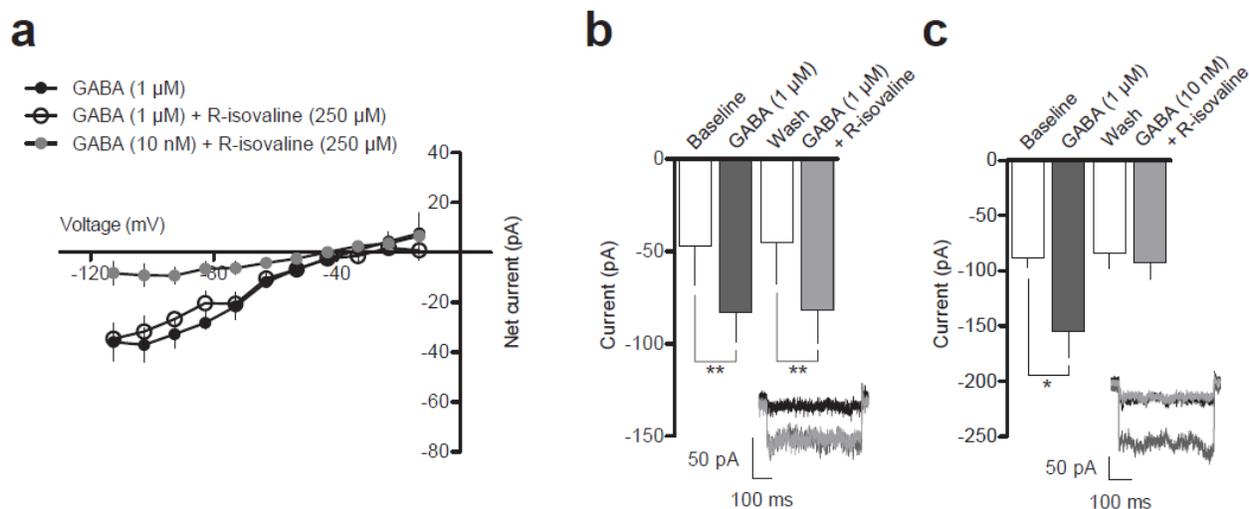


**Figure 2.3 GABA and baclofen, but not isovaline, evoke inwardly rectifying currents in transfected AtT-20 cells.**

a) Baseline subtracted IV curve for GABA and GABA + CGP 52432 ( $n = 7$ ). Inset shows example currents at baseline (black), in the presence of GABA (dark grey) or in the presence of GABA + CGP 52432 (light grey). b) Baseline subtracted IV curve for baclofen ( $n = 6$ ). Inset shows example currents at baseline (black) or in the presence of baclofen (dark grey). c) Baseline subtracted IV curves for R-isovaline at 3 concentrations ( $50 \mu\text{M}$   $n = 6$ ;  $250 \mu\text{M}$   $n = 8$ ;  $1 \text{ mM}$   $n = 4$ ). d) Example currents at baseline (black) and on application of 3 concentrations of R-isovaline (dark grey). e) Baseline subtracted IV curve for S-isovaline ( $n = 4$ ). Inset shows example currents at baseline (black) or in the presence of S-isovaline (dark grey). f) Summary graph showing the currents recorded at  $-112.5 \text{ mV}$  at baseline (open bars) or on application of GABA, R-baclofen, R-isovaline or S-isovaline (shaded bars). Data are represented as mean  $\pm$  SEM. \*\*\* =  $p < 0.001$ .

### **R-isovaline did not alter responses to other GABA<sub>B</sub> receptor agonists**

To test if R-isovaline could modulate GABA action, I applied GABA (1  $\mu$ M) in the presence or absence of R-isovaline (250  $\mu$ M). As shown in Figure 2.4 a,b there was no statistically significant difference in the currents with application of GABA alone ( $-82.9 \pm 16.3$  pA) compared to GABA + R-isovaline ( $-81.5 \pm 18.1$  pA, repeated measures 1-way ANOVA with Bonferroni's post hoc test,  $F_{(3,2)} = 24.41$ ,  $p > 0.05$ ,  $n = 3$ ). To determine if R-isovaline is a positive modulator, GABA was applied at 1/100 of an effective concentration in the presence of R-isovaline after confirming a response to a high concentration of GABA. As shown in Figure 2.4 a,c, co-application of R-isovaline with a low concentration of GABA (10 nM) did not significantly change the measured current as determined by repeated measures 1-way ANOVA with Bonferroni's post hoc test (GABA + R-isovaline  $-92.7 \pm 15.0$  pA vs. wash  $-84.3 \pm 13.4$  pA,  $F_{(3,5)} = 6.131$ ,  $p > 0.05$ ,  $n = 6$ ). R-isovaline did not positively modulate or occlude GABA-induced inward currents.

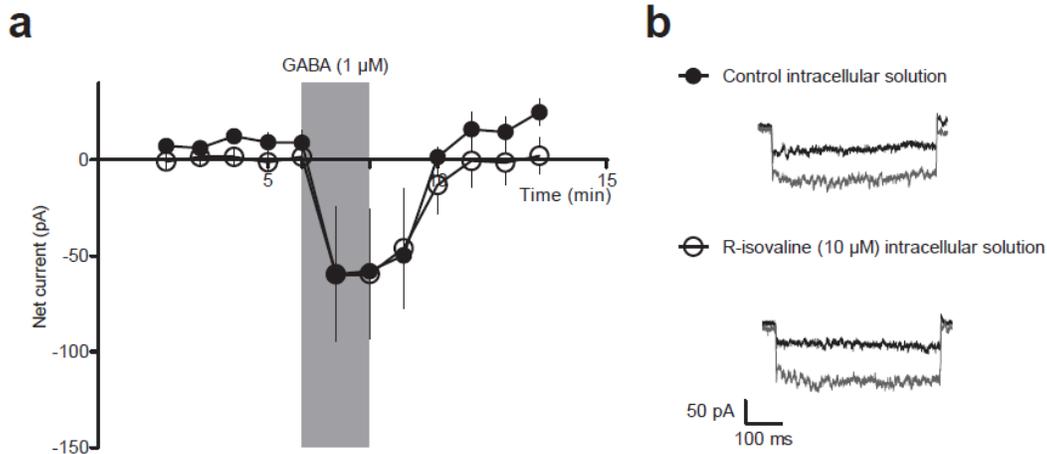


**Figure 2.4 R-isovaline does not modulate GABA-evoked currents in transfected AtT-20 cells.**

a) Baseline subtracted IV curves for GABA (1  $\mu$ M; n=3) and co-application of R-isovaline with GABA (1  $\mu$ M n = 3; 10 nM n = 6). b) Graph shows that R-isovaline does not alter the current evoked by a high concentration of GABA. Inset shows example currents at baseline (black), on application of GABA (1  $\mu$ M; dark grey) and on co-application of 1  $\mu$ M GABA with 250  $\mu$ M R-isovaline (light grey). c) Graph shows that R-isovaline does not alter the current when co-applied with a low concentration of GABA. Inset shows example currents at baseline (black), on application of 1  $\mu$ M GABA (dark grey), and on co-application of 10 nM GABA with 250  $\mu$ M R-isovaline (light grey). Data are expressed as mean  $\pm$  SEM. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ .

To investigate the possibility that R-isovaline could only induce a current by an intracellular action, I applied R-isovaline via the intracellular pipette solution to AtT-20 cells while measuring the GABA-evoked inward current. It was assumed that the concentration of R-isovaline that would transport across the cell membrane would be lower than that applied extracellularly. Therefore, I used a lower concentration for these experiments (10  $\mu$ M). There was no difference in the currents recorded with electrodes containing R-isovaline or control solution as determined by a 2-way ANOVA (main effect of R-isovaline  $F_{(1, 105)} = 1.77$ ,  $p = 0.2$ ,  $n = 6$  for both electrode groups, Figure 2.5 a,b). The difference in the GABA-evoked current recorded with

electrodes containing R-isovaline or control solution was not statistically significantly different (control  $-59.5 \pm 33.6$  pA vs. R-isovaline  $-58.0 \pm 17.7$  pA, Bonferroni's post hoc test,  $p > 0.05$ ).



**Figure 2.5 Intracellular R-isovaline does not alter baseline or GABA-mediated currents in transfected AtT-20 cells.**

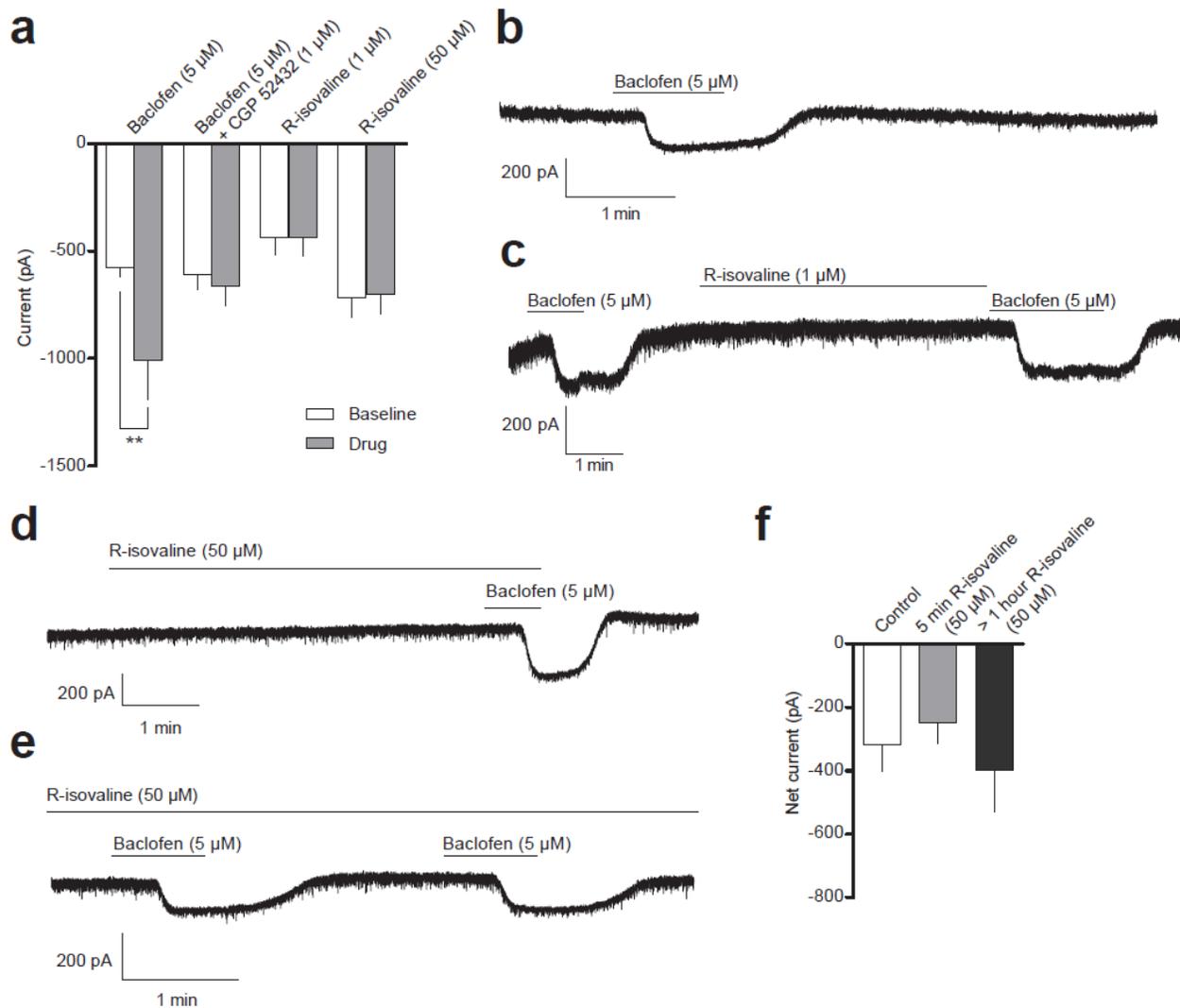
a) Baseline subtracted current at  $-112.5$  mV over time for control intracellular solution ( $n = 6$ ) and intracellular solution containing R-isovaline ( $10 \mu\text{M}$ ;  $n = 6$ ). b) Example currents at baseline (black) or during  $1 \mu\text{M}$  GABA application (dark grey) recorded with electrodes containing control solution (top) or intracellular solution containing R-isovaline (bottom). Data represent mean  $\pm$  SEM.

### **R-isovaline did not alter GABA<sub>B</sub> mediated K<sup>+</sup> currents in cultured hippocampal neurons**

One caveat with expression systems is that transfected receptor subunits may not associate with auxiliary proteins, scaffolding proteins or signal transduction pathways that exist in neurons that natively express GABA<sub>B</sub> receptors. Therefore, I tested the effects of R-isovaline in cultured hippocampal neurons, examining membrane currents at a holding potential of  $-80$  mV

in a high  $K^+$  extracellular solution to enhance inward  $K^+$  currents. Cultured hippocampal neurons were chosen due to their well characterised response to GABA<sub>B</sub> receptor agonists (Sodickson and Bean, 1996). Baclofen (5  $\mu$ M) induced reversible inward currents in all neurons tested and was determined to be significant by a repeated measures 2-way ANOVA (baclofen  $-1005 \pm 187$  pA vs. baseline  $-547 \pm 48$  pA, interaction  $F_{(3, 21)} = 4.01$ ,  $p < 0.02$ , Bonferroni's post hoc test,  $p < 0.01$ ,  $n = 7$ , Figure 2.6 a,b). The currents evoked by baclofen were antagonised by 1  $\mu$ M CGP 52432 (baclofen + CGP 52432  $-665 \pm 88$  pA vs. baseline  $-607 \pm 70$  pA,  $p > 0.05$ ,  $n = 7$ , Figure 2.6 a). R-isovaline (1  $\mu$ M or 50  $\mu$ M; 5 min (Cooke et al., 2009)) did not produce an observable change in membrane current in this preparation (1  $\mu$ M R-isovaline  $-434 \pm 86$  pA vs. baseline  $-438 \pm 81$  pA,  $p > 0.05$ ,  $n = 3$ ; 50  $\mu$ M R-isovaline  $-701 \pm 92$  pA vs. baseline  $-716 \pm 94$  pA,  $p > 0.05$ ,  $n = 8$ , Figure 2.6 a,c).

Finally, I assessed if R-isovaline could alter the response to baclofen in cultured hippocampal neurons. Application of 50  $\mu$ M R-isovaline for 5 min (Figure 2.6 d,f) in high  $K^+$  extracellular solution prior to co-application with baclofen did not alter the baclofen evoked current compared to the current evoked by baclofen in control extracellular solution (control  $-316 \pm 84$  pA,  $n = 8$ ; 5 min R-isovaline pretreatment  $-249 \pm 66$  pA,  $n = 4$ ). To test if a longer incubation with R-isovaline was required, I incubated hippocampal cells in their culture dish with 50  $\mu$ M R-isovaline in ACSF at room temperature for over 1 hour prior to transfer to the bath for patch clamp recording. All solutions during recording also contained 50  $\mu$ M R-isovaline. Prolonged incubation with R-isovaline also failed to affect the baclofen evoked current (> 1 hour R-isovaline  $-400 \pm 130$  pA,  $n = 8$ , Figure 2.6 e,f). Analysis with a 1-way ANOVA found no significant difference between any of the treatment groups ( $F_{(2,17)} = 0.40$ ,  $p = 0.68$ ).



**Figure 2.6 R-isovaline does not activate GABA<sub>B</sub> receptors in cultured hippocampal neurons.**

a) Current at a holding potential of -80 mV in a high extracellular K<sup>+</sup> solution at baseline (open bars) and on application of baclofen (n = 7), baclofen + CGP 52432 (n = 7) and R-isovaline at low (1  $\mu$ M; n = 3) and high (50  $\mu$ M; n = 8) concentrations (shaded bars). b) Example shows baclofen-mediated current c) Example shows lack of effect of low concentration of R-isovaline in a cell that responded to baclofen both before and after R-isovaline application. d) Example shows the effect of baclofen after 5 min pretreatment with 50  $\mu$ M R-isovaline. e) Example shows the effects of 2 applications of baclofen after prolonged incubation (> 1 hour) with R-isovaline. f)

Graph shows the magnitude of the current evoked by 5  $\mu$ M baclofen in control extracellular solution (n = 8), after 5 minutes pretreatment with R-isovaline (n = 4) or after > 1 hour pretreatment with R-isovaline (n = 8). Data represent mean  $\pm$  SEM. \*\* = p < 0.01.

## Discussion

### **R-isovaline did not mimic the actions of the other GABA<sub>B</sub> agonists**

Our studies show that R-isovaline did not induce an inward current in AtT-20 cells heterologously expressing GABA<sub>B</sub> receptors or in cultured hippocampal neurons natively expressing GABA<sub>B</sub> receptors. While GABA and baclofen activated GABA<sub>B</sub> receptors, co-application with R-isovaline did not occlude or modulate GABA or baclofen responses. The data are in contrast to reports that show R-isovaline-mediated activation of GABA<sub>B</sub> receptors (Cooke et al., 2012; Whitehead et al., 2012). However, others have demonstrated that isovaline does not alter the postsynaptic electrical properties of hippocampal pyramidal neurons (Shin et al., 2011b).

Mouse pituitary AtT-20 cells contain endogenous GIRK channels (Dousmanis and Pennefather, 1992), a common effector channel of GABA<sub>B</sub> receptors (Padgett and Slesinger, 2010). In the present experiments, AtT-20 cells transiently transfected with GABA<sub>B</sub> receptor subunits responded to GABA or baclofen with an inwardly rectifying current that reversed near the calculated equilibrium potential for K<sup>+</sup>. GABA<sub>B</sub> receptors mediated the inward currents as confirmed by antagonism with CGP 52432. Baclofen also induced a GABA<sub>B</sub> receptor-dependent inward current in cultured hippocampal neurons, attributable to activation of GIRK channels (Sodickson and Bean, 1996).

## **Potential reasons for the lack of action of R-isovaline in our assay system**

In contrast to the GABA- or R-baclofen-induced activation of endogenously expressed GIRK channels, R-isovaline did not produce a response in either cell type. Previous work has demonstrated that R-isovaline induces a large increase in GABA<sub>B</sub>-mediated K<sup>+</sup> conductance in thalamocortical neurons of brain slices, albeit with slower response kinetics than with baclofen (Cooke et al., 2009, 2012). One explanation for the difference may be that R-isovaline only acts at a specific isoform of the GABA<sub>B</sub> receptor. GABA<sub>B</sub> receptors are obligate heterodimers consisting of a GABA<sub>B1</sub> and GABA<sub>B2</sub> subunit (Jones et al., 1998a; Kaupmann et al., 1998; White et al., 1998; Thuault et al., 2004). While there is only one isoform of the GABA<sub>B2</sub> subunit, 2 isoforms of the GABA<sub>B1</sub> subunit are functionally expressed in the mammalian CNS (Bettler and Tiao, 2006). If R-isovaline interacted selectively with GABA<sub>B</sub> receptors containing the GABA<sub>B1b</sub> subunit, I would not observe an effect because AtT-20 cells were transfected with cDNA coding only for the GABA<sub>B1a</sub> subunit. However, GABA<sub>B1b</sub> subunits differ only on the N terminus, a region that has been shown not to affect ligand binding or receptor function (Galvez et al., 2000a; Bettler et al., 2004; Hannan et al., 2012). There are no known pharmacological or functional differences between GABA<sub>B1a</sub>- or GABA<sub>B1b</sub>- subunit containing GABA<sub>B</sub> receptors upon heterologous expression, thus, a selective action at a specific isoform of the GABA<sub>B1</sub> subunit is unprecedented (Bettler et al., 2004; Bettler and Tiao, 2006).

Another explanation for the failure of isovaline to activate GABA<sub>B</sub> receptors in our studies is that its action could depend on an alternative protein that associates with the GABA<sub>B</sub> receptor or signalling components. GABA<sub>B</sub> receptors can display pharmacological and functional

differences depending on cell type and subcellular location. This is due to variation in cell specific proteins that regulate GABA<sub>B</sub> receptors or their responses (Couve et al., 2001; Perroy et al., 2003; Balasubramanian et al., 2007; Fowler et al., 2007; Kanaide et al., 2007; Labouèbe et al., 2007; Pinard et al., 2010; Schwenk et al., 2010). Cooke et al. (2012) suggested a cell-specific mechanism of action in thalamic slices because significantly fewer neurons responded to R-isovaline than to R-baclofen; in addition, a subset of neurons responded to baclofen but not R-isovaline (Cooke et al., 2012). Candidate cell specific proteins include potassium channel tetramerization domain (KCTD) proteins, which directly associate with GABA<sub>B</sub> receptors to alter ligand affinity and response kinetics such as desensitisation (Schwenk et al., 2010). However, differential expression of KCTD proteins may not explain a lack of effect of R-isovaline on K<sup>+</sup> currents in hippocampal pyramidal neurons because the hippocampus has been reported to express all 3 KCTD proteins that act as GABA<sub>B</sub> auxiliary subunits (Schwenk et al., 2010). Alternatively, specific RGS proteins that alter GABA<sub>B</sub> receptor efficacy (Fowler et al., 2007; Labouèbe et al., 2007) may be required for R-isovaline to have sufficient efficacy to evoke GABA<sub>B</sub>-mediated GIRK currents. It also is possible that isovaline acts as a biased agonist (Martí-Solano et al., 2013) at GABA<sub>B</sub> receptors in such a way that it does not influence the membrane delimited coupling of the GABA<sub>B</sub> receptor to GIRK channels. Instead, isovaline may initiate GABA<sub>B</sub>-mediated signalling cascades that indirectly influence membrane conductances, for example activation of Src-kinases which may not have been present in the preparations used (Diversé-Pierluissi et al., 1997; Callaghan et al., 2008).

Isovaline's effects, particularly in the CNS, may depend on cellular location. For example, isovaline has anti-epileptic properties which are not likely GABA<sub>B</sub>-mediated, but instead are

postulated to result from a selective enhancement of hippocampal interneuronal activity by non-synaptically increasing inhibitory input and/or eliciting a shunting phenomenon onto pyramidal neurons (Shin et al., 2011b; Yu et al., 2014). Our experiments demonstrated no effect of isovaline on hippocampal pyramidal neurons, but do not exclude isovaline actions on interneurons.

### **Summary and future directions**

In summary, these studies demonstrate that R-isovaline does not activate GABA<sub>B</sub>-mediated GIRK currents in AtT-20 cells or isolated hippocampal pyramidal neurons. Furthermore, intracellular or extracellular application of R-isovaline does not occlude or modulate the actions of other GABA<sub>B</sub> receptor agonists in these isolated cell systems. Future studies should be aimed at determining if cell specific modulators of GABA<sub>B</sub> receptor signalling are required for isovaline-induced K<sup>+</sup> currents in thalamic and other responsive neurons.

## Chapter 3 GABA<sub>B</sub> modulation of dopamine release in the NAc core

### Introduction

A critical part of the neural circuitry mediating motivated behaviours comprises VTA dopamine neurons and their projections to the NAc. A change in the firing pattern of VTA dopamine neurons from tonic to burst firing results in increased dopamine release (Zhang *et al.*, 2009a). Goal-directed behaviours are coupled to phasic increases in the concentration of dopamine released from terminals (Gan *et al.*, 2010; Sugam *et al.*, 2012). Therefore, factors that influence extracellular dopamine concentration ( $[DA]_o$ ) in the NAc play an important role in shaping behaviour.

In addition to reuptake via dopamine transporters which are highly expressed in the NAc (Nirenberg *et al.*, 1997a), there are several mechanisms regulating  $[DA]_o$ . ACh from cholinergic interneurons enhances release of dopamine by activating nACh receptors on dopaminergic nerve terminals (Zhou *et al.*, 2001). Conversely, inhibition of dopamine release can occur by activation of presynaptic D2 dopamine receptors (Schmitz *et al.*, 2002) or  $\kappa$ -opioid receptors (Britt and McGehee, 2008) located on dopamine nerve terminals.  $\mu$ -opioid receptors are expressed on cholinergic interneurons and decrease  $[DA]_o$  by inhibiting ACh release (Britt and McGehee, 2008). During a pulse train stimulation, activation of ionotropic glutamate AMPA receptors on MSNs inhibits dopamine release via retrograde action of  $H_2O_2$  at dopamine terminals, whereas, activation of ionotropic GABA<sub>A</sub> receptors on MSNs opposes this effect (Avshalumov *et al.*, 2003). Interestingly, inhibition of AMPA or GABA<sub>A</sub> receptors does not modulate single-pulse evoked  $[DA]_o$  in striatal slices (Chen *et al.*, 2006), making this an ideal

preparation for exploring the direct modulation of terminal dopamine release (Zhang and Sulzer, 2012; Ferris et al., 2013).

GABA<sub>B</sub> receptors are expressed in both the VTA and the NAc (Bowery et al., 1987; Ciccarelli et al., 2012). While the effects of activation of GABA<sub>B</sub> receptors on dopamine cell bodies in the VTA are well characterised (Johnson and North, 1992; Cruz et al., 2004), the role that NAc GABA<sub>B</sub> receptors have in modulating terminal dopamine release requires further characterisation. *In vivo* studies have demonstrated that reverse dialysis of baclofen, a GABA<sub>B</sub> receptor agonist, in the NAc decreases dopamine release (Xi et al., 2003). However, it is unknown if this is due to a direct effect on dopamine neuron nerve terminals. Therefore, I measured electrically stimulated dopamine release in ventral striatal slices using fast scan cyclic voltammetry.

Several studies demonstrate that baclofen can reduce drug seeking behaviours in humans and rodent models of addiction (Shoptaw et al., 2003; Slattery et al., 2005; Halbout et al., 2011).

One consideration for the pharmacological treatment of drug addiction is whether drugs of abuse can alter the effectiveness of therapeutic agents such as baclofen. Therefore, I also tested if GABA<sub>B</sub>-mediated alterations in dopamine release were modulated by chronic cocaine exposure.

## **Materials and methods**

### **Subjects**

Male C57BL/6 mice (2-3 months), provided by the University of British Columbia breeding facility or Jackson Laboratory (Sacramento, CA), were maintained on a 12 hour light dark cycle

(7 am – 7pm) with access to food and water *ad libitum*. Male mice were used to exclude cyclic effects of estradiol and progesterone on dopamine concentration (Becker, 1999). All experiments were performed in the light cycle. All experimental protocols were in accordance with the Canadian Council on Animal Care.

### **Slice preparation**

Mice were anaesthetised with isoflurane, decapitated and the brain tissue extracted into ice-cold sucrose solution containing (in mM) sucrose (75), NaCl (87), KCl (2.5), NaH<sub>2</sub>PO<sub>4</sub> (1.25), NaHCO<sub>3</sub> (25), MgCl<sub>2</sub> (7) and CaCl<sub>2</sub> (0.95). Coronal slices containing the NAc core were cut 250 µm thick using a vibratome (Leica, Nussloch, Germany). Slices were incubated in artificial cerebral spinal fluid (ACSF) containing (in mM) NaCl (119), KCl (1.6), NaH<sub>2</sub>PO<sub>4</sub> (1.0), NaHCO<sub>3</sub> (26.2), MgCl<sub>2</sub> (1.4), CaCl<sub>2</sub> (2.4) and glucose (11) at 31.5°C for at least 45 minutes before being transferred to a bath (1 mL volume) constantly perfused with the aid of a peristaltic pump (1.67 mL/min, General Electric health care) with the same ACSF at 31.5°C for voltammetry recordings. All solutions were continuously saturated with 95% O<sub>2</sub>/5% CO<sub>2</sub>.

### **Cocaine administration**

Mice (2 months, 22 – 30 g) were divided into groups and habituated to locomotor activity boxes for 30 minutes. The following day (day 0) all groups were given a single 0.9 % saline injection (10 µL/g, i.p.). For the next 5 days mice were given either daily i.p. injections of 15 mg/kg cocaine HCl or the equivalent volume of saline. Locomotor activity was recorded for 30 minutes each day immediately after the injection using ANY-maze software V 4.81 (Stoelting, Wood Dale, Illinois). In order to test whether withdrawal was necessary for alterations in NAc GABA<sub>B</sub> receptors, slices were prepared for voltammetry at 2 time points representing either no

withdrawal (24 hours after the final injection) or withdrawal (10 -12 days after the final injection).

### **Fast-scan cyclic voltammetry**

Dopamine release in the NAc core was measured using fast scan cyclic voltammetry. Dopamine release was evoked using electrical stimulation applied with a two pronged tungsten bipolar stimulating electrode (1.5 m $\Omega$  resistance) positioned flush with the tissue for local surface stimulation. The electrical stimulation was either a single monophasic rectangular pulse (2 ms duration, 300  $\mu$ A current) or the same pulse applied 2 or 5 times at varying frequencies (10, 40 or 100 Hz). The higher frequency stimulations were designed to mimic burst firing of dopamine neurons (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Ferris et al., 2013). Dopamine release evoked with these stimulations is sensitive to tetrodotoxin, suggesting that dopamine release evoked with this stimulations is dependent upon successful invasion of dopamine neuron terminals by action potentials (Cragg, 2003). Carbon fibre microelectrodes (tip length of 150 - 180  $\mu$ m) were prepared by pulling glass electrodes containing 7  $\mu$ m diameter carbon fibres (Goodfellow, Coraopolis, Pennsylvania, USA). The voltammetric electrode was positioned between the two tips of the stimulating electrode with the aid of a binocular microscope and then lowered 50 – 100  $\mu$ m into the tissue. A triangular waveform (from -0.4 to +1.0 V vs. Ag/AgCl, 400 V/s scan rate) was applied at 10 Hz to induce the oxidation and reduction of dopamine at the surface of the carbon fibre electrode. The evoked currents were attributed to dopamine when the corresponding voltammogram featured a characteristic shape with peak oxidation and reduction potentials (vs. Ag/AgCl) at approximately +600 and -200 mV, respectively. To determine the time course of dopamine release and uptake, the current at the

peak oxidation ( $\sim +600$  mV) was plotted against time. Carbon fibre electrodes were calibrated post hoc using ACSF containing  $1 \mu\text{M}$  dopamine. Recordings were made every 5 minutes unless otherwise stated. Once a stable baseline for peak dopamine release had been achieved (4 consecutive recordings within 10% of each other) drugs were bath applied for 10 or 60 min. The time point of 10 minutes was chosen based on electrophysiological studies in slices in which the maximum effect of baclofen occurred within 5 minutes (Cruz et al., 2004; Cooke et al., 2012). For paired pulse experiments, dopamine release was electrically evoked every 2.5 min alternating between a single pulse (P1) or 2 pulses 10 ms apart; (P1+P2 at 100 Hz, Cragg, 2003; Zhang and Sulzer, 2004). Once the peak values for both P1 and P1+P2 had stabilised, the current elicited by P1 was subtracted from the current elicited during P1+P2 to determine the current attributable to P2 (i.e.,  $P1+P2 - P1 = P2$ ). The paired pulse ratio was reported here as P2/P1.

## **Drugs**

Stock solutions of baclofen HCl (R-enantiomer only, Sigma, Oakville, Ontario, Canada) and CGP 52432 (Tocris, Minneapolis, Minnesota, USA) were made with double distilled  $\text{H}_2\text{O}$  and stored at  $4^\circ\text{C}$ . Quinpirole HCl (Tocris) and mecamylamine HCl (Sigma) stock solutions were prepared with double distilled  $\text{H}_2\text{O}$  then aliquoted and frozen. Dopamine (3-hydroxytyramine-HCl, Sigma) stock was made up with Na-metabisulfite (Fisher, Ottawa, Ontario, Canada) daily. Drugs were made to their final concentration in oxygenated ACSF on the day of experimentation. Cocaine HCl (Sigma) was made to a final concentration with 0.9% saline and stored at  $4^\circ\text{C}$  for no longer than a week.

## Statistical analysis

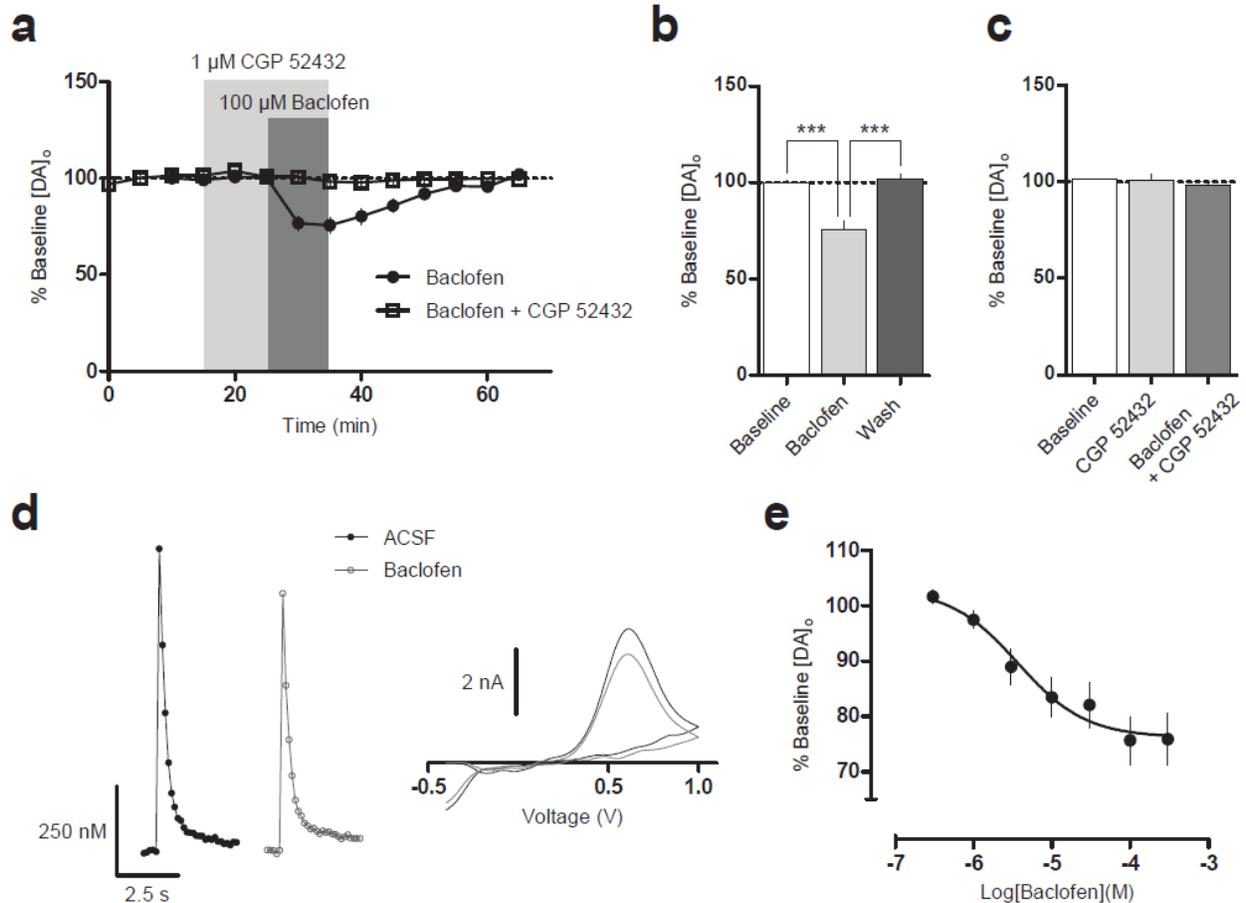
Peak baseline dopamine release for each slice was determined by averaging the peak current of the 4 consecutive recordings before drug application. All recordings were then expressed as a percentage of the baseline value. To assess changes in dopamine reuptake rate, a 1.5 s section of current vs. time was plotted from 0.2s after the peak and the time constant ( $\tau$ ) of the decay was measured using GraphPad Prism V. 5 (La Jolla, California, USA) by fitting a single exponential curve. To determine the maximal uptake rate ( $V_{max}$ ) I used the equation  $d[DA]/dt = -V_{max}/((K_m/[DA]) + 1)$  where  $K_m$  has been experimentally determined to be 0.2  $\mu$ M and  $d[DA]/dt$  was measured as the slope of the initial (linear) portion of the dopamine decay curve between points where the concentration of dopamine released was equivalent for baseline and baclofen treatment (Near et al., 1988; Wightman et al., 1988; Wightman and Zimmerman, 1990). For electrochemistry experiments, “n” refers to the number of recordings (maximum one per slice) from at least 3 mice, expressed as number of recordings/number of mice. A power analysis using the equation  $n = [2 * (\text{standard deviation})^2 * (\text{power index})] / (\text{change in mean})^2$ , where the power index equals 10.5 for  $\alpha$  (significance level) = 0.05 and  $\beta$  (chance of type II error) = 0.1, indicated that to detect a minimum 10% change in effect with a standard deviation of 5%, a minimum of 5 slices were needed per experiment. Unless otherwise indicated, data met the assumptions of equal variances. To test if the response to baclofen followed a Gaussian distribution data from multiple experiments in which 100  $\mu$ M baclofen was applied for 10 minutes, in the absence of other drugs, was compiled and tested for normality. A total of 34 values from 5 separate experiments were used for the analysis. The D’Agostino and Pearson omnibus normality test determined that the results obtained for baclofen-mediated

inhibition of dopamine release did not deviate from a normal distribution ( $K2 = 1.882$ ,  $p = 0.3902$ ). Consequently, parametric statistics were used for all analyses. Final data is expressed as mean  $\pm$  SEM between recordings or for the locomotor activity data between animals. Paired or unpaired t-tests and one or two-way ANOVAs with Bonferroni's post-hoc test were performed as appropriate using GraphPad Prism V. 5. A p value of  $< 0.05$  was considered statistically significant.

## Results

### Activation of GABA<sub>B</sub> receptors inhibits dopamine release in the NAc core

To determine if baclofen could modify  $[DA]_o$  recorded at the carbon fibre electrode after electrical stimulation of dopamine release, baclofen was bath applied to ventral striatal slices that contained the NAc core. Baclofen (100  $\mu$ M; 10 min) reduced single-pulse evoked  $[DA]_o$  to  $76 \pm 4$  % of baseline, an effect that was completely washed out after 30 min (baseline:  $100 \pm 0.9$  %, wash:  $102 \pm 2$  %  $p < 0.001$ , repeated measures one-way ANOVA,  $F_{(2,8)} = 23.02$ ,  $n = 9/3$ ; Figure 3.1 a,b,d). Pre-treatment of the GABA<sub>B</sub> receptor antagonist, CGP 52432 (1  $\mu$ M; 10 min) alone did not significantly alter  $[DA]_o$  and blocked the baclofen-mediated suppression of  $[DA]_o$  in the NAc core (baseline:  $101 \pm 0.3$  %, CGP 52432:  $101 \pm 3$  %, baclofen + CGP 5234:  $98 \pm 2$  %,  $p > 0.05$ , repeated measures one-way ANOVA,  $F_{(2,5)} = 1.04$ ,  $n = 6/3$ ; Figure 3.1 a,c). The effect of baclofen was concentration-dependent with an  $IC_{50}$  of  $3.7 \pm 0.4$   $\mu$ M (1 concentration per slice, 0.3  $\mu$ M,  $n = 3/3$ ; 1  $\mu$ M,  $n = 11/3$ ; 3  $\mu$ M,  $n = 5/3$ ; 10  $\mu$ M,  $n = 6/3$ ; 30  $\mu$ M,  $n = 9/3$ ; 100  $\mu$ M,  $n = 9/3$ ; 300  $\mu$ M,  $n = 10/6$ , Figure 3.1 e). The data summarized in Figure 3.1 b,c suggest that baclofen acts at GABA<sub>B</sub> receptors to suppress  $[DA]_o$ . Further, single-pulse electrical stimulation does not evoke sufficient endogenous GABA release to modulate GABA<sub>B</sub> receptors.

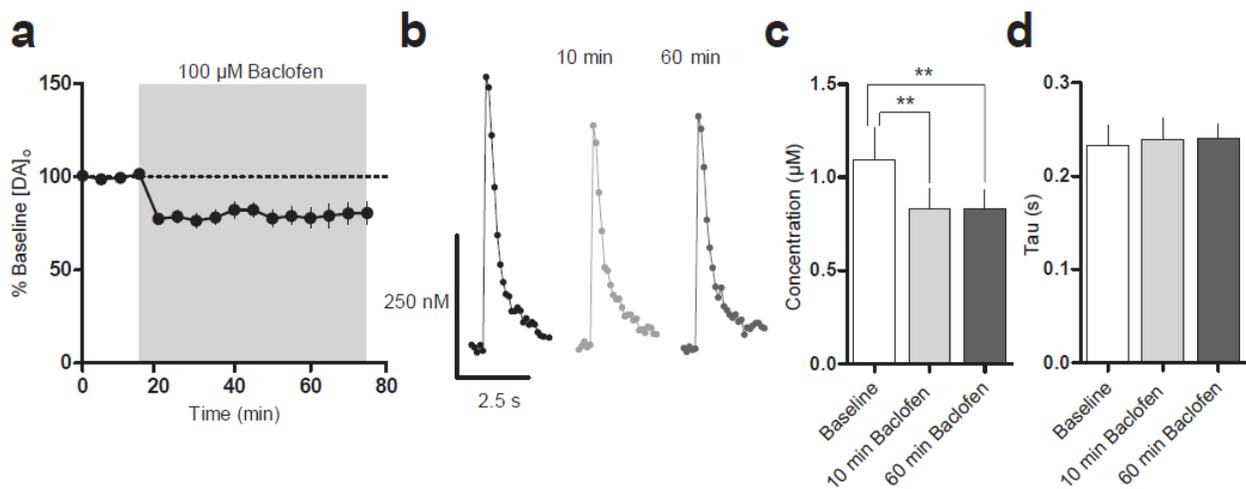


**Figure 3.1 Baclofen suppresses dopamine release evoked by a single pulse stimulus.**

a) Baclofen (100  $\mu$ M, 10 min,  $n = 9$  recordings from 3 animals = 9/3, filled circles) suppresses evoked dopamine release in NAc core slices. This effect is blocked when baclofen is administered with the GABA<sub>B</sub> antagonist, CGP 52432 ( $n = 6/3$ , open squares). b) Bar graph demonstrating dopamine release before (open bar) during (light shaded bar) and after washout of baclofen (100  $\mu$ M, dark shaded bar). c) Bar graph demonstrating dopamine release before (open bar), during CGP 52432 application alone (1  $\mu$ M, light shaded bar) or during co-application of CGP 52432 with baclofen (dark shaded bar). d) Example concentration vs. time plots with corresponding voltammograms depicting the concentration of dopamine released at baseline (black line) or after a 10 minute application of 100  $\mu$ M baclofen (grey line). e) Concentration response curve for baclofen (0.3 – 300  $\mu$ M,  $n = 3 - 11$ ). Bars represent mean  $\pm$  S.E.M. \*\*\* =  $p < 0.001$ .

Pharmacotherapy with baclofen likely requires sustained suppression of dopamine release. To assess if the effect of baclofen changed over time, I bath applied 100  $\mu$ M baclofen to slices for

60 min. Maximal baclofen-mediated suppression of single pulse-evoked  $[DA]_o$  after 10 min application was not significantly different to baclofen-mediated suppression after 60 min application (baseline:  $1.1 \pm 0.2 \mu\text{M}$ ; baclofen<sub>10 min</sub>:  $0.8 \pm 0.1 \mu\text{M}$ ; baclofen<sub>60 min</sub>:  $0.8 \pm 0.1 \mu\text{M}$ ,  $p > 0.05$ , repeated measure one way ANOVA,  $F_{(2,7)} = 9.72$ ,  $n = 8/3$ ; Fig. 3.2 a-c), indicating that prolonged application of a saturating baclofen concentration maintains its efficacy at suppressing  $[DA]_o$ .



**Figure 3.2 Baclofen-mediated suppression of evoked dopamine release does not desensitise over time.**

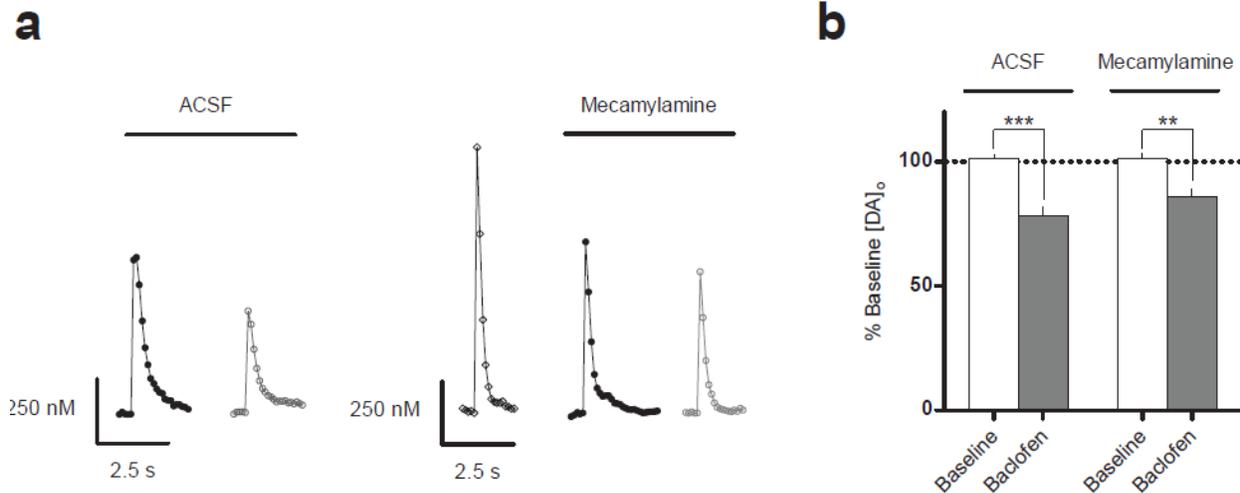
a) Baclofen-mediated suppression of evoked dopamine release does not desensitise with 1 hour exposure to baclofen ( $100 \mu\text{M}$ ). b) Example concentration vs. time plots of evoked dopamine release in the absence (black line), or in the presence of baclofen at 10 (light grey line) or 60 min (dark grey line). c) Bar graph of the concentration of evoked dopamine release in the absence (open bar) or presence of baclofen at 10 (light shaded bar) or 60 min (dark shaded bar) ( $n = 8/3$ ). d) Average tau in the absence (open bar) or presence of baclofen at 10 or 60 min ( $n = 8/3$ ). Bars represent mean  $\pm$  S.E.M. \*\* =  $p < 0.01$ .

Next, I assessed if baclofen-mediated suppression of  $[DA]_o$  was due to a reduction in dopamine release, or an increase in dopamine reuptake. The time constant of the decay of the current,

represented by tau, has been demonstrated to be positively correlated with  $K_m$ , suggesting that  $\tau$  is an appropriate measurement of dopamine uptake (Yorgason et al., 2011). Current vs. time plots of evoked dopamine indicated that baclofen (10 or 60 min) did not significantly alter the decay of the current ( $\tau_{\text{baseline}} = 0.2 \pm 0.02$  s,  $\tau_{\text{baclofen 10 min}} = 0.2 \pm 0.02$  s;  $\tau_{\text{baclofen 60 min}} = 0.2 \pm 0.01$  s;  $p > 0.05$ , repeated measures one-way ANOVA,  $F_{(2,7)} = 0.19$ ,  $n = 8/3$ ; Figure 3.2d).

Furthermore,  $V_{\text{max}}$  was not significantly changed in the presence of baclofen (baseline:  $2.2 \pm 0.1$   $\mu\text{M/s}$ , baclofen<sub>10min</sub>:  $2.1 \pm 0.1$   $\mu\text{M/s}$ ; baclofen<sub>60min</sub>:  $2.0 \pm 0.2$   $\mu\text{M/s}$ ;  $p > 0.05$ , repeated measures one-way ANOVA,  $F_{(2,7)} = 1.34$ ,  $n = 8/3$ ).

Previous studies have found that antagonists for glutamate, GABA, adenosine, opioid and cannabinoid receptors have no effect on single-pulse evoked dopamine release (Zhang and Sulzer, 2012). However, the electrical stimulation used to evoke dopamine release in the ventral striatum also evokes ACh release that enhances dopamine release via a rapid action at nACh receptors (Zhou et al., 2001). Thus, baclofen-mediated suppression of dopamine release may be indirect, due to an effect of GABA<sub>B</sub> receptors on cholinergic interneurons in the NAc. If this hypothesis is correct, the effect of baclofen should be occluded by preventing ACh action on nACh receptors. I blocked nACh receptors by bath applying mecamylamine (100  $\mu\text{M}$ ) for 30 minutes, resulting in an inhibition of single-pulse evoked dopamine release to  $50 \pm 5$  % of baseline (Figure 3.3a). Subsequently, in the presence of mecamylamine, baclofen (100  $\mu\text{M}$ ) was bath applied for 10 minutes. Analysis with a two-way ANOVA revealed no effect of mecamylamine on baclofen-mediated suppression of dopamine release (baclofen:  $78 \pm 1$  % of baseline,  $n = 7/3$ ; baclofen and mecamylamine:  $86 \pm 3$  % of baseline,  $n = 7/3$ ;  $p > 0.05$ , repeated measures two-way ANOVA,  $F_{(1, 12)} = 2.06$ ; Figure 3.3 a,b).



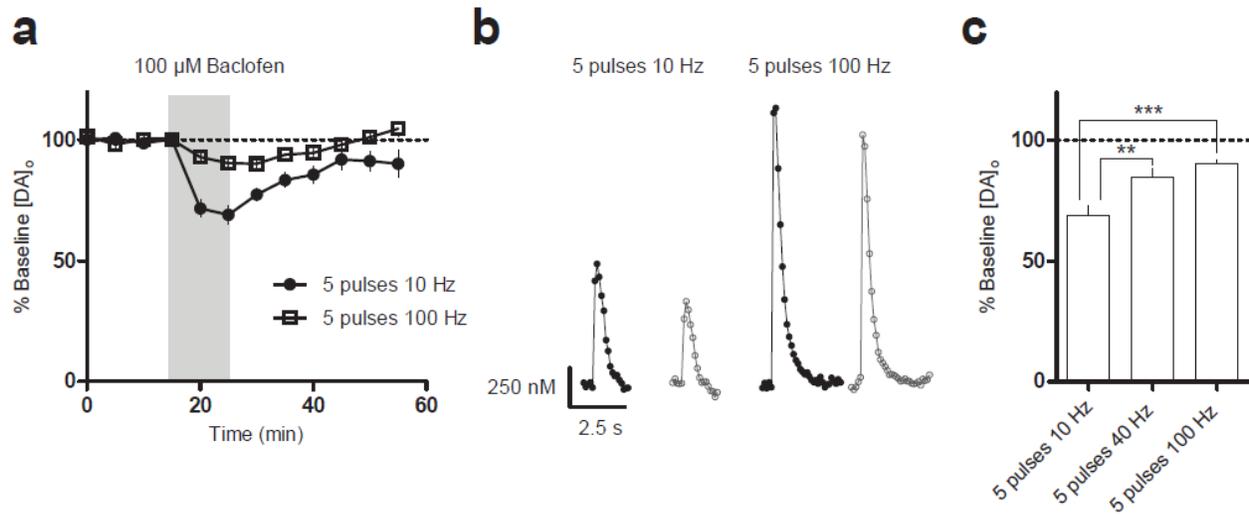
**Figure 3.3 Baclofen-mediated suppression of evoked dopamine release does not require cholinergic input.**

a) Example concentration vs. time plots before (black line, filled circles) and after (grey line, open circles) baclofen application in artificial cerebral spinal fluid (ACSF) (left panel) or co-applied mecamylamine (right). An example trace from before application of mecamylamine is shown with open diamonds. b) Bar graphs demonstrating the effect of 100  $\mu$ M baclofen (shaded bars) compared to baseline (open bars) on evoked dopamine release in the absence (left;  $n = 7/3$ ) or presence of 100  $\mu$ M mecamylamine (right:  $n = 7/3$ ). Data are expressed as mean  $\pm$  S.E.M. \*\*\* =  $p < 0.001$ . \*\* =  $p < 0.01$ .

### Baclofen-mediated suppression of dopamine release is frequency-dependent

To assess the frequency-dependence of baclofen-mediated suppression of  $[DA]_o$ , I tested the effect of baclofen on dopamine release evoked with 5 pulses at 10, 40 or 100 Hz. Interestingly, baclofen-mediated suppression was inversely proportional to the frequency at which dopamine release was evoked. At 10 Hz, a stimulation mimicking tonic firing of dopamine neurons, baclofen (100  $\mu$ M, 10 min) inhibited  $[DA]_o$  to  $69 \pm 12$  % of baseline ( $n = 9/3$ ), which was significantly different from the magnitude of baclofen-mediated inhibition at 100 Hz, a much

higher frequency than typical dopamine neuron burst firing ( $90 \pm 4\%$  of baseline,  $n = 9/4$ ,  $p < 0.001$ , one way ANOVA,  $F_{(2,24)} = 13.59$ , Figure 3.4 a-c). The inhibitory effect of baclofen on dopamine evoked at 40 Hz, a stimulation mimicking burst firing of dopamine neurons (inhibited release to  $85 \pm 3\%$  of baseline, Figure. 3.4 c), was blocked with CGP 52432 ( $1 \mu\text{M}$ , 10 min) (baseline:  $100 \pm 1\%$ , CGP 52432:  $101 \pm 5\%$ , baclofen + CGP 52432:  $101 \pm 4\%$ ,  $n = 9/3$ ,  $p > 0.05$ , repeated measures one-way ANOVA,  $F_{(2,8)} = 0.233$ ).



**Figure 3.4 Baclofen inhibits dopamine in a frequency dependent manner.**

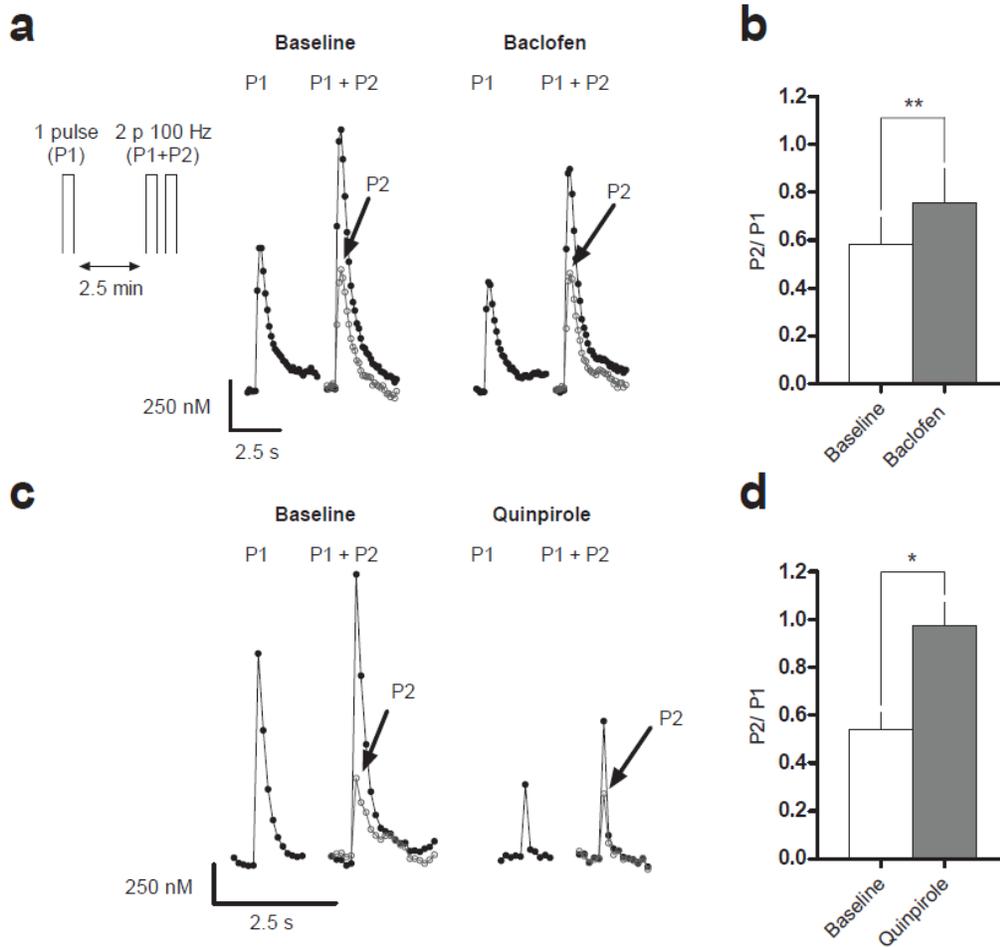
a) The time course of baclofen ( $100 \mu\text{M}$ ) on dopamine release electrically evoked with a 5 pulse 10 Hz (filled circles,  $n = 9/3$ ) or a 5 pulse 100 Hz stimulus (open squares;  $n = 9/4$ ). b) Example concentration vs. time plots depicting dopamine release evoked with 5 pulse stimuli at 10 Hz (left) or 100 Hz (right) in the absence (black line) or presence (grey line) of  $100 \mu\text{M}$  baclofen (10 min). c) Bar graph demonstrating the maximal effect of baclofen (10 min,  $100 \mu\text{M}$ ) on dopamine release evoked by differing stimulation protocols ( $n = 9/3-4$  for all bars). Data is expressed as mean  $\pm$  S.E.M, \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

To further explore the mechanism by which baclofen inhibits  $[\text{DA}]_o$ , I investigated if baclofen alters the probability of dopamine release. Short-term plasticity occurs by altering calcium

entry or hyperpolarising nerve terminals resulting in residual  $\text{Ca}^{2+}$ , which then promotes neurotransmitter release to subsequent stimulations in close succession (Cragg, 2003; Zhang and Sulzer, 2004, 2012). Interventions that decrease the probability of neurotransmitter release produce a higher paired pulse ratio of neurotransmitter release (Cragg, 2003; Zhang & Sulzer, 2004, 2012). I measured the effect of baclofen on the paired pulse ratio using a protocol described in Cragg, 2003 and Zhang & Sulzer, 2004. A single pulse stimulation (termed P1) was followed 2.5 minutes later by a 2 pulse stimulation with an interpulse interval of 10 ms (P1+P2). The current attributable to the second pulse of the 2 pulse stimulus (P2) was determined by subtracting the current elicited by the single pulse stimulus from the current elicited by the 2 pulse stimulation (i.e.,  $P1+P2 - P1 = P2$ ). The magnitude of P2 was then expressed as a fraction of P1 ( $P2/P1$ , i.e., the paired pulse ratio). While this frequency of stimulation is higher than firing frequencies typically associated with dopamine neuron burst firing, this protocol has previously been used as an effective way to measure the short term plasticity of dopamine release and how this plasticity is altered by dopamine release modulating drugs (Hyland et al., 2002; Cragg, 2003; Zhang & Sulzer, 2004).

Consistent with previous work (Zhang & Sulzer, 2004), I observed a basal paired-pulse depression of dopamine release (Figure 3.5). However, after baclofen application (100  $\mu\text{M}$ ), the paired-pulse ratio was significantly increased (baseline  $P2/P1$ :  $0.6 \pm 0.1$  vs. baclofen  $P2/P1$ :  $0.8 \pm 0.1$ ,  $p < 0.01$ , paired t-test,  $t = 3.51$ ,  $df = 10$ ,  $n = 11/4$ ; Figure 3.5 b,d). Consistent with a decrease in release probability, application of quinpirole (300 nM), which activates D2 presynaptic autoreceptors to inhibit evoked dopamine release (Schmitz *et al*, 2003), increased the paired-pulse ratio (baseline  $P2/P1$ :  $0.5 \pm 0.07$  vs. quinpirole  $P2/P1$ :  $1 \pm 0.1$ ,  $p < 0.05$ , paired

t-test,  $t = 3.58$ ,  $df = 6$ ,  $n = 7/3$ ; Fig. 5C, D). Notably, dopamine release at P1 was significantly less with quinpirole than baclofen (quinpirole<sub>10min</sub>:  $32 \pm 6$  % of baseline vs. baclofen<sub>10min</sub>:  $75 \pm 4$  % of baseline,  $p < 0.001$ , 2-way ANOVA with bonferroni post hoc  $F_{(8,141)} = 8.95$ , Figure 3.5 a).



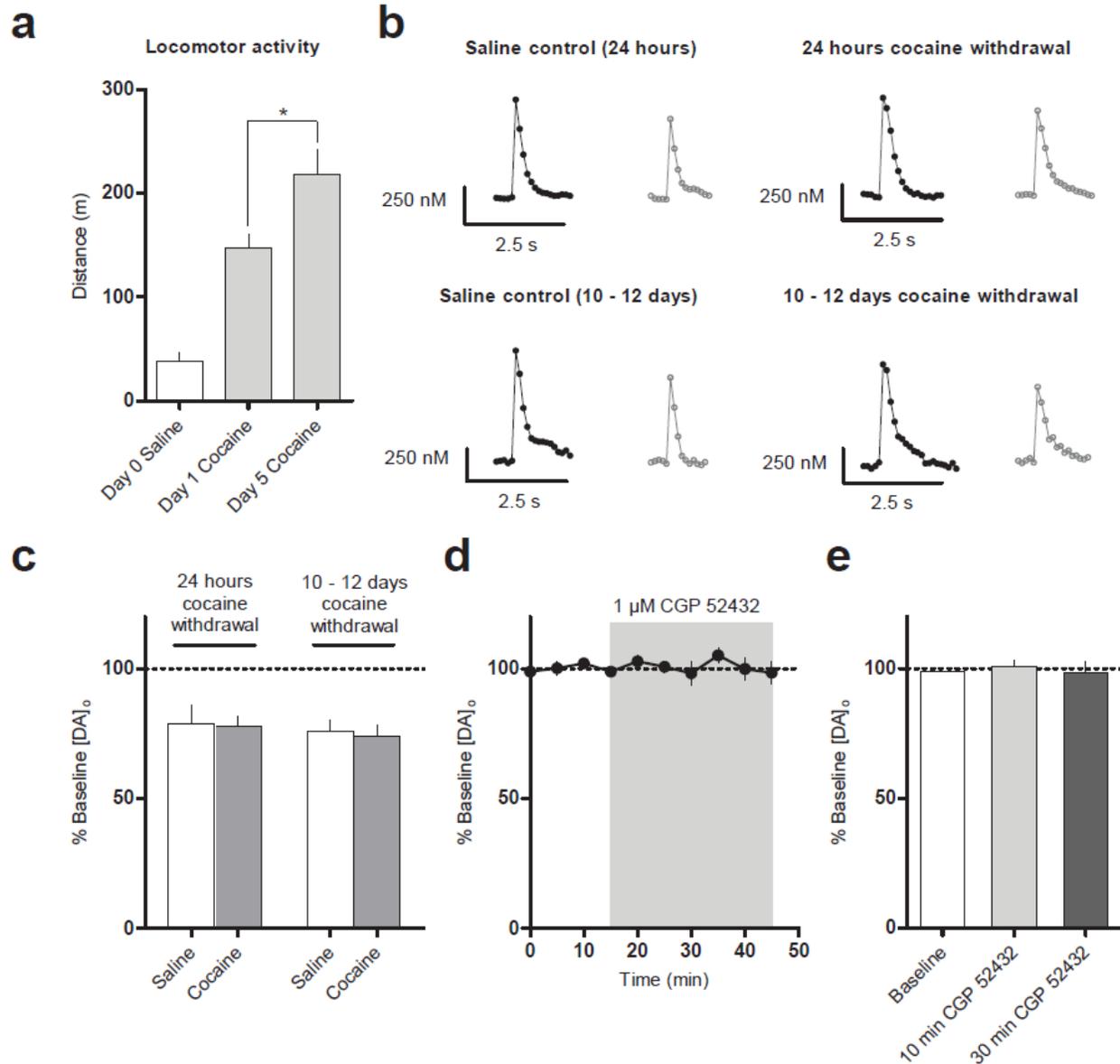
**Figure 3.5 Baclofen increases the paired pulse ratio of dopamine release.**

a) Example concentration vs. time plots depicting dopamine release evoked by a single pulse stimulus (P1) or a 2 pulse 100 Hz stimulus (P1+P2, black lines) and the calculated dopamine release attributable to the 2<sup>nd</sup> pulse of the 2 pulse stimulus (P2, grey lines) under baseline conditions or in the presence of 100  $\mu$ M baclofen. Inset, diagram of the stimulation protocols used. b) As in A) but with 300 nM quinpirole. c) Peak dopamine release attributable to P2 divided by peak dopamine release evoked by P1 before (open bar) and during (shaded bar) 100  $\mu$ M baclofen ( $n = 11/4$ ) or 300 nM quinpirole application ( $n = 7/3$ ). Bars represent mean  $\pm$  S.E.M, \* =  $p < 0.05$ , \*\* =  $p < 0.01$ . \*\*\* =  $p < 0.001$ .

## **Baclofen-induced suppression of dopamine release persists in cocaine-sensitised mice**

Baclofen has been tested as a therapeutic agent for cocaine addiction (Shoptaw et al., 2003). Therefore, I determined if chronic cocaine treatment altered baclofen-mediated suppression of dopamine release. As shown in Figure 3.6 a, locomotor activity in mice immediately following cocaine administration (15 mg/kg, i.p.) was significantly greater after 5 daily injections compared to the first day (distance travelled day 1:  $147 \pm 13$  m vs. day 5:  $218 \pm 25$  m,  $p < 0.05$ , one way ANOVA,  $F_{(2,15)} = 28.49$ ,  $n = 6$ ), demonstrating that these mice exhibited locomotor sensitisation to cocaine. In slices containing the NAc core taken 24 hours after the final cocaine injection, baclofen (100  $\mu$ M, 10 min) inhibited single pulse-evoked dopamine release to  $78 \pm 4$  % ( $n = 7/3$ ) of baseline while the suppression of dopamine release in slices from saline injected mice was  $79 \pm 7$  % of baseline ( $n = 4/3$ ; Figure 3.6 b,c). Baclofen-mediated suppression of  $[DA]_o$  was similar after 10 – 12 days withdrawal from cocaine or saline (cocaine:  $74 \pm 5$  % of baseline ( $n = 6/3$ ) vs. saline:  $76 \pm 4$  % of baseline ( $n = 6/3$ ), Figure 3.6 b,c). Analysis with two-way ANOVA showed no effect of cocaine treatment ( $F_{(1,19)} = 0.44$ ,  $p > 0.05$ ) or withdrawal period ( $F_{(1, 19)} = 0.02$ ,  $p > 0.05$ ). To determine if evoked GABA could alter  $GABA_B$ -mediated suppression of dopamine after withdrawal from cocaine treatment, I bath applied a  $GABA_B$  antagonist to slices taken from mice 10-12 days after the final cocaine injection. CGP 52432 (1  $\mu$ M, 10 or 30 min) did not significantly alter evoked  $[DA]_o$  (baseline:  $99 \pm 1$  %, CGP 52432<sub>10 min</sub>:  $101 \pm 2$  %, CGP 52432<sub>30 min</sub>:  $98 \pm 4$  %, repeated measures one-way ANOVA,  $F_{(2,3)} = 0.14$ ,  $n = 4/3$ ; Figure 3.6 d,e) suggesting withdrawal from chronic cocaine treatment does not increase endogenous GABA

tone at GABA<sub>B</sub> receptors on dopamine terminals in slices. Taken together, these results suggest that baclofen-mediated suppression of dopamine release persists with chronic cocaine treatment.



**Figure 3.6 Baclofen-mediated suppression of dopamine release persists with sensitising cocaine treatment.**

a) Distance travelled by mice during 30 minutes immediately following i.p. cocaine (15 mg/kg; shaded bars) or saline (0.9%; open bar) injections (n = 6). b) Example concentration vs. time plots of evoked dopamine in the presence (grey lines) or absence (black line) of 100 μM

baclofen after saline (left panel) or cocaine (right panel) treatment. c) Effect of 100  $\mu$ M baclofen (10 min) on dopamine release evoked by a single pulse stimulus after chronic saline (open bars) or cocaine treatment (shaded bars) at 24 hours or 10 – 12 days withdrawal (n = 4 – 7/3). d) Time course of CGP 52432 on single pulse evoked dopamine release from mice withdrawn from chronic cocaine treatment (10 – 12 days, n = 4/3). E) Bar graph compares maximal evoked dopamine release before (open bar) as well as 10 (light shaded bar) and 30 min (dark shaded bar) after CGP 52432 application. Data is expressed as mean  $\pm$  S.E.M, \* = p < 0.05.

## Discussion

Baclofen has been proposed as a potential therapeutic for anti-craving and the maintenance of abstinence in cocaine addicts (Tyacke et al., 2010). I report here that baclofen acts at presynaptic GABA<sub>B</sub> receptors on dopamine neurons in the NAc core to inhibit terminal dopamine release. Furthermore, baclofen-mediated inhibition of dopamine release in the NAc was resistant to desensitisation for up to one hour bath application and was not altered by a sensitising cocaine regimen, supporting the notion that GABA<sub>B</sub> receptors are promising targets for addiction treatments.

### **Activation of GABA<sub>B</sub> receptors on dopamine terminals suppresses dopamine release in the NAc core**

Our data support the hypothesis that baclofen acts at GABA<sub>B</sub> receptors directly on dopamine terminals to inhibit dopamine release. Firstly, baclofen concentration-dependently inhibited dopamine release via activation of GABA<sub>B</sub> receptors in the NAc core. These results are consistent with the ability of baclofen to inhibit extracellular striatal dopamine concentration *in vivo* (Smolders et al., 1995) and evoked dopamine in the caudate putamen in mouse brain slices (Schmitz et al., 2002). GABA<sub>B</sub> agonists do not alter the membrane properties of MSNs in the NAc suggesting that they do not express postsynaptic GABA<sub>B</sub> receptors (Uchimura and North,

1991; Molnár et al., 2009). In contrast, GABA<sub>B</sub> agonists can inhibit the release of GABA and glutamate onto MSNs, indicating that there are pre-synaptic GABA<sub>B</sub> auto- and hetero-receptors on the terminals of GABAergic and glutamatergic neurons (Uchimura and North, 1991; Molnár et al., 2009). It has been shown that antagonists for GABA and glutamate do not modulate dopamine release evoked by a single pulse stimulus in slices (Chen et al., 2006; Zhang and Sulzer, 2012), indicating that a single pulse electrical stimulus does not cause sufficient GABA or glutamate release to modify dopamine release (Zhang and Sulzer, 2012). Therefore, this preparation is an ideal way to determine whether drugs are able to act directly on dopamine terminals. In agreement with other literature using fast scan cyclic voltammetry in striatal brain slices (Chen et al., 2006), a single pulse stimulus was not sufficient to evoke endogenous GABA to act at GABA<sub>B</sub> receptors, as a GABA<sub>B</sub> antagonist alone had no effect on terminal dopamine release.

Secondly, baclofen continued to suppress dopamine release in the presence of a nACh receptor antagonist. Tonically active cholinergic interneurons in the striatum contribute to increased [DA]<sub>o</sub> (Zhou et al., 2001). Our results support the hypothesis that baclofen does not act at these cholinergic interneurons to reduce electrically evoked dopamine release by inhibiting ACh release. This is consistent with *in vivo* microdialysis studies that demonstrate minimal effects of baclofen on ACh release in the NAc (Anderson et al., 1993; DeBoer and Westerink, 1994).

Thirdly, similar to the D2 agonist quinpirole, baclofen reduces the probability of dopamine release, suggesting a mechanistic overlap. Both D2 receptors and GABA<sub>B</sub> receptors couple to G<sub>αi/o</sub> proteins, inhibit VGCCs, and open GIRK channels (Cardozo and Bean, 1995; Beckstead et

al., 2004; Cruz et al., 2004). Presynaptic GABA<sub>B</sub> receptors can inhibit neurotransmitter release by a rapid inhibitory effect on voltage-gated calcium entry into nerve terminals (Cardozo and Bean, 1995; Laviv et al., 2011). Notably, the magnitude of paired pulse facilitation induced by baclofen was less than that induced by quinpirole. Because the paired pulse ratio is dependent on the release probability of the first pulse (Zhang *et al.*, 2009b), the difference observed is likely due to the efficacy of quinpirole at inhibiting single pulse-evoked DA release compared to baclofen. A direct effect of GABA<sub>B</sub> receptor activation on dopamine release is supported by ultrastructural evidence of GABA<sub>B</sub> receptors expressed on dopamine terminals (Charara et al., 2000), which is presumably similar between monkeys and rodents.

### **Frequency-dependence of dopamine release**

Midbrain dopamine neurons fire in a slow irregular fashion resulting in tonic release of dopamine. However, in response to salient environmental stimuli, dopamine neuron action potentials are clustered into bursts and the increase in [DA]<sub>o</sub> in the projection areas is much larger than that observed for regularly spaced trains of action potentials at the same frequency (Grace and Bunney, 1984a, 1984b; Tepper et al., 1995; Overton and Clark, 1997, Figure 1.1). Consistent with this idea, electrical stimulation mimicking burst firing increases [DA]<sub>o</sub> (Hyland *et al.*, 2002; Zhang *et al.*, 2009b; Ferris *et al.*, 2013). A 10 Hz stimulation frequency mimics tonic firing of dopamine neurons while a 40 Hz stimulation frequency mimics burst firing of dopamine neurons. The 100 Hz stimulation frequency used in these experiments is much higher than what would likely happen physiologically and was intended as an experimental maximal stimulation frequency. Baclofen-mediated suppression of dopamine release was inversely proportional to the stimulation frequency, such that suppression of dopamine release evoked

with a low frequency stimulus was significantly greater compared to suppression of dopamine evoked by burst stimuli. With a similar impact on tonic vs. burst firing to  $\mu$ -opioids and nicotine (Zhang and Sulzer, 2004; Britt and McGehee, 2008), one might expect a modification in signal-to-noise ratio to enhance perception of important environmental stimuli (Couey *et al.*, 2007; Zhang *et al.*, 2009b). However, baclofen applied to the VTA decreases dopamine neuron activity in slices (Chen *et al.*, 2005) and when given systemically, baclofen decreases the average firing rate and bursts of dopamine neurons *in vivo* (Erhardt *et al.*, 2002). Therefore, because baclofen reduces DA burst firing and terminal DA release, the net effect of baclofen in the mesolimbic system is likely an overall suppression of dopamine outflow. Consistent with this, systemic baclofen decreases drug induced increases in dopamine concentration in the NAc measured with *in vivo* microdialysis (Fadda *et al.*, 2003; Fu *et al.*, 2012). Based on this, I speculate that baclofen reduces perception of salient stimuli, which may contribute to its role in reducing the reinforcing properties of addictive drugs (Vlachou and Markou, 2010).

### **GABA<sub>B</sub>-mediated suppression of dopamine release persists with chronic cocaine treatment**

A reduction in baclofen-mediated inhibition following cocaine exposure could have implications for the pharmacotherapy of addiction. Thus, it was of interest whether a sensitising cocaine regimen induced changes in GABA<sub>B</sub> receptor-modulation of dopamine release at terminals in the NAc. Previous work has implicated increased GABAergic transmission in the NAc during cocaine self-administration (Wydra *et al.*, 2013) or after withdrawal from chronic cocaine administration (Xi *et al.*, 2003; Wydra *et al.*, 2013). While it has been hypothesized that cocaine-induced increases in GABAergic transmission may alter GABA<sub>B</sub> receptor number or function (Xi

et al., 2003), there is no consensus on whether GABA<sub>B</sub> receptors are altered with chronic cocaine treatment or withdrawal. For example, Frankowska et al., (2008) reported that GABA<sub>B</sub> receptor binding was decreased immediately after chronic cocaine treatment and Xi et al. (2003) found that baclofen stimulated GABA<sub>B</sub> receptor activation was reduced after 3 weeks of withdrawal from chronic cocaine. However, another study reported that GABA<sub>B</sub> receptor activation was not altered immediately after chronic cocaine treatment (Kushner and Unterwald, 2001). Our results demonstrate that GABA<sub>B</sub>-mediated suppression of dopamine release was not altered 24h or 10-12 days after chronic cocaine treatment.

Discrepancies of previous findings may be due to the inability to establish whether the reduction in GABA<sub>B</sub> receptor binding/activation was ubiquitous among different neuronal inputs or cell populations within the NAc or due to changes at selective neuronal inputs. Using slice voltammetry, I was able to differentiate between GABA<sub>B</sub> activation on dopamine terminals as opposed to alterations of GABA<sub>B</sub> receptor function on glutamatergic or GABAergic terminals. Because a saturating dose of baclofen suppressed dopamine in the NAc to a similar extent in chronic cocaine or saline treated mice, it is unlikely that chronic cocaine treatment or withdrawal altered the number or function of GABA<sub>B</sub> receptors on dopamine terminals. Notably, while Xi et al. (2003) demonstrated that withdrawal from cocaine increased GABA in the NAc, there were no changes in basal dopamine or baclofen-induced inhibition of either glutamate or DA (Xi et al., 2003). These results suggest that while chronic cocaine may increase endogenous GABA, GABA uptake or degradation mechanisms may be sufficient to prevent GABA reaching concentrations which could produce agonist induced desensitisation of GABA<sub>B</sub> receptors on dopamine terminals, the mechanism hypothesised by Xi *et al*, (2003). Conversely,

the mechanisms which result in desensitisation or down regulation of GABA<sub>B</sub> receptors may be less efficient at presynaptic GABA<sub>B</sub> receptors on dopamine neuron terminals. Indeed, our results support the latter hypothesis, because the response to prolonged application of a saturating concentration of baclofen did not desensitise, resulting in a sustained suppression of dopamine release.

### **Summary and future directions**

Together these data suggest that baclofen inhibits dopamine release in the NAc core via action at GABA<sub>B</sub> receptors on dopamine terminals. Baclofen-mediated inhibition of dopamine release persisted with a sensitising cocaine treatment. Although drugs of abuse have different mechanisms of action, a common feature is that they all interact with the mesolimbic dopamine system to increase terminal dopamine concentration (Di Chiara and Imperato, 1988). The magnitude of dopamine release in the NAc scales with the value of a reward or anticipated value of a forthcoming reward which helps shape reward-learning behaviour (Gan et al., 2010). The suppression of dopamine release in the NAc, regardless of cocaine treatment, suggests a potential mechanism for baclofen's utility as an anti-craving compound. While the effect of baclofen was sustained for up to an hour in slices, in future studies it will be important to determine whether chronic systemic administration of baclofen alters the ability of baclofen to decrease terminal dopamine release as well as whether the concentration-response relationship differs or GABA<sub>B</sub> receptors are desensitised or down regulated inequitably on different dopaminergic, GABAergic or glutamatergic inputs to the NAc.

## **Chapter 4 Presynaptic regulation of dopamine release by GABA<sub>B</sub> or D2 receptors in the NAc core is unaltered by access to a cafeteria diet.**

### **Introduction**

Dopamine release in the NAc is important in mediating motivated behaviours for rewards such as palatable food (Salamone et al., 1990; Bassareo and Di Chiara, 1999; Peciña et al., 2003; Roitman, 2004). In return, over consumption of high fat food has been shown to alter dopamine signalling, resulting in hypofunction of the mesolimbic dopamine system. This has been demonstrated by decreased extracellular dopamine concentration in the NAc (Geiger et al., 2009), reduced responding for and decreased the motivation to obtain food or drugs of abuse (Davis et al., 2008; Geiger et al., 2009; Shin et al., 2011a; Speed et al., 2011) and elevated reward thresholds (Johnson and Kenny, 2010).

The behavioural effects of dopamine are dependent upon the concentration of dopamine released from dopaminergic nerve terminals (Phillips et al., 2003; Oleson et al., 2012; Sugam et al., 2012). Dopamine neurons fire tonically at low frequencies but when presented with salient stimuli dopamine neurons fire in high frequency bursts resulting in increased dopamine release (Grace and Bunney, 1984a, 1984b; Chergui et al., 1994; Floresco et al., 2003; Venton et al., 2003; Zhang et al., 2009a; Zweifel et al., 2009). The phasic increases in dopamine concentration are important for guiding behaviours (Phillips et al., 2003; Oleson et al., 2012; Sugam et al., 2012; Saunders et al., 2013).

In addition to dopamine neuron firing patterns, NAc dopamine concentration is under the control of both dopamine reuptake and presynaptic regulation of neurotransmitter release

(Chergui et al., 1994; Schmitz et al., 2002; Floresco et al., 2003; Venton et al., 2003; Zhang et al., 2009a; Pitman et al., 2014). Changes in either of these could contribute to hypofunction of the mesolimbic dopamine system associated with diet induced obesity. Dopamine reuptake is mediated by dopamine transporters which transport extracellular dopamine back into the nerve terminal (Rickhag et al., 2013). Dopamine release has been shown to be regulated by both dopamine, acting at D2 autoreceptors (Schmitz et al., 2002), and the major inhibitory neurotransmitter GABA, acting at GABA<sub>B</sub> receptors (Charara et al., 2000; Pitman et al., 2014). D2 and GABA<sub>B</sub> agonists suppress dopamine release by a direct effect at receptors located on dopaminergic nerve terminals (Schmitz et al., 2002; Pitman et al., 2014).

Obesity and/or access to a high fat diet can modify terminal dopamine concentration by multiple mechanisms. Diet induced obesity can decrease dopamine transporter binding (South and Huang, 2008). The expression of D2 receptors may also be altered by diet-induced obesity. However, the effects of obesity on D2 receptor expression are inconsistent, such that decreased D2 receptor expression (Hajnal et al., 2008; van de Giessen et al., 2013), increased D2 expression (Huang et al., 2005; South and Huang, 2008; Sharma and Fulton, 2013), or no changes in D2 receptor expression (van de Giessen et al., 2012) have all been reported in the NAc. It has yet to be determined whether obesity-induced alterations in D2 receptor expression result in altered regulation of dopamine release by D2 receptors. Likewise, there have been no studies examining whether diet can modify suppression of dopamine release mediated by presynaptic GABA<sub>B</sub> receptors on dopaminergic nerve terminals.

I determined whether diet induced obesity could alter the regulation of NAc core extracellular dopamine concentration by 3 mechanisms; changing dopamine transporter activity; altering presynaptic D2 receptor function; and/or altering GABA<sub>B</sub>-mediated inhibition of dopamine release. To examine diet-induced obesity mediated effects, I chose a cafeteria diet, which induces rapid weight gain over 6-7 weeks and has previously been demonstrated to decrease D2 receptor expression in the striatum (Johnson and Kenny, 2010).

## **Methods**

### **Cafeteria diet**

All experimental designs were approved by the University of Calgary Animal Care Committee and were in accordance with the Canadian Council on Animal Care. P60 Long Evans rats (Charles River Laboratory) were maintained on a 12 hour reverse light-dark cycle. Rats were divided into 3 groups matched for weight. One group (chow only) received ad libitum standard laboratory rat chow (Prolab<sup>®</sup> RMH 2500 5P14, St. Louis, Missouri). The second group (extended access) received ad libitum laboratory rat chow as well as ad libitum access to a palatable cafeteria diet. The final group (restricted access) received ad libitum access to chow and 1 hour access to the cafeteria diet every day beginning at 2 hours after the start of the dark cycle. Based on published methods (Johnson and Kenny, 2010) cafeteria diet was given in 2 different protocols in order to examine the effects of access to palatable food that resulted in diet-induced obesity (extended access) in comparison with access that did not result in increased weight gain (restricted access). The cafeteria diet consisted of smooth peanut butter (Kraft<sup>®</sup>), Froot Loops<sup>®</sup> (Kellogg's<sup>®</sup>), nacho cheese Doritos<sup>®</sup> (FritoLay<sup>®</sup>), Hot Dogs (Kirkland<sup>®</sup>) and chocolate flavoured Timbits<sup>®</sup> (Tim Hortons<sup>®</sup>). The nutrient compositions of all foods are summarised in Table 4.1

The quantity of food consumed was measured daily between 2 – 4 hours after the onset of the dark cycle. Rats were also weighed each day during this time. Rats were maintained on their respective diet for between 40 – 60 days. Tail blood was removed from a subset of rats before access to the cafeteria diet and at 40 days of access. To determine if access to the cafeteria diet altered the feeding related hormones, insulin and leptin, the concentration of these hormones were measured before access to the cafeteria diet, as well as at day 40 of the diet using enzyme-linked immunosorbent assay kits (Crystal Chem, Downers Grove, Illinois) following manufactures protocols.

**Table 4.1 Nutrient composition of cafeteria diet food**

|                                  | <b>Peanut Butter</b> | <b>Froot Loops®</b> | <b>Doritos®</b> | <b>Hog Dogs</b> | <b>Timbits®</b> | <b>Chow</b> |
|----------------------------------|----------------------|---------------------|-----------------|-----------------|-----------------|-------------|
| <b>kCal/g</b>                    | 6.0                  | 4.1                 | 5.2             | 3.0             | 3.7             | 3.0         |
| <b>% kCal from Fat</b>           | 72                   | 8                   | 46              | 78              | 38              | 12          |
| <b>% kCal from Protein</b>       | 12                   | 4                   | 5               | 17              | 6               | 29          |
| <b>% kCal from Carbohydrates</b> | 16                   | 88                  | 50              | 5               | 56              | 59          |

### **Fast-scan cyclic voltammetry**

Coronal brain slices containing the NAc core were prepared and voltammetry experiments undertaken as previously described (Pitman et al., 2014). Rats were anaesthetised with isoflurane and then decapitated. The brain was removed from the skull and placed into an ice cold slicing solution containing (in mM) sucrose (75), NaCl (87), KCl (2.5), NaH<sub>2</sub>PO<sub>4</sub> (1.25), NaHCO<sub>3</sub> (25), MgCl<sub>2</sub> (7), CaCl<sub>2</sub> (0.95) and kynurenic acid (2) saturated with 95% O<sub>2</sub>/5% CO<sub>2</sub>. A vibratome (Leica, Nussloch, Germany) was used to cut 250 µm slices which were immediately places in artificial cerebrospinal fluid (ACSF) containing (in mM) NaCl (119), KCl (1.6), NaH<sub>2</sub>PO<sub>4</sub> (1.0), NaHCO<sub>3</sub> (26.2), MgCl<sub>2</sub> (1.4), CaCl<sub>2</sub> (2.4) and glucose (11) warmed to 31.5°C and saturated

with 95% O<sub>2</sub>/5% CO<sub>2</sub>. Slices were incubated in this solution for at least 45 minutes before recording.

For fast scan cyclic voltammetry experiments slices were placed in a recording chamber and were constantly perfused with ACSF (1.67 mL/min). Glass microelectrodes with carbon fibres (7 µm diameter, Goodfellow, Coraopolis, Pennsylvania, USA) were prepared to expose a final carbon fibre tip length of 150 - 180 µm. A bipolar stimulating electrode was used to electrically evoke localised dopamine release with a single pulse stimulus (1 monophasic rectangular pulse, 2 ms duration, 300 µA current). Since the magnitude of D2 or GABA<sub>B</sub> receptor inhibition of dopamine release is reduced when dopamine release is evoked by multiple pulse stimulations applied at high frequencies compared with low frequency simulations (Zhang and Sulzer, 2004; Pitman et al., 2014) all effects were examined with a single pulse stimulus that enables observations of a maximal effect of D2 or GABA<sub>B</sub> receptor agonists. The carbon fibre electrode was placed approximately 50 – 100 µm into the tissue, between the 2 tips of the stimulating electrode. Oxidation and reduction of dopamine at the surface of the carbon fibre electrode was achieved by voltage ramps from -0.4 to +1.0 to -0.4 V (vs. Ag/AgCl ground electrode, 400 V/s scan rate, 10 Hz). Dopamine was identified by a characteristic voltammogram with peak oxidation and reduction potentials (vs. Ag/AgCl) at approximately +600 and -200 mV, respectively. Dopamine release was evoked every 5 minutes and the peak current at the peak oxidation potential (~+600 mV) was recorded. Once 4 consecutive recordings with less than 10% variation had been reported (the average of which was used to determine “baseline” dopamine release), R-baclofen HCl was applied for 10 minutes, then washed off for a further 30 minutes. Quinpirole was applied for 45 minutes in order to confirm a stabilisation of effect.

Data were converted to concentrations by post-hoc calibration of the electrode with 10  $\mu\text{M}$  dopamine. Data were expressed as extracellular evoked dopamine concentration  $[\text{DA}]_o$  as a percent of baseline  $[\text{DA}]_o$  (% baseline  $[\text{DA}]_o$ ).

The time constant of decay or the evoked dopamine currents ( $\tau$ ), which has been demonstrated to be positively correlated with  $K_m$  (Yorgason et al., 2011), was determined by analysing the current recorded during the first 1.5s after the peak with a non-linear regression analysis (GraphPad Prism V. 5, La Jolla, California, USA). The maximal uptake rate ( $V_{\max}$ ) was determined by using the equation  $d[\text{DA}]/dt = -V_{\max}/((K_m/[\text{DA}]) + 1)$  where  $K_m$  has been experimentally determined to be 0.2  $\mu\text{M}$  and  $d[\text{DA}]/dt$  was measured as the slope of the initial (linear) portion of the DA decay curve at concentrations greater than 1  $\mu\text{M}$  (Near et al., 1988; Wightman et al., 1988; Wightman and Zimmerman, 1990).

## Drugs

R-baclofen HCl (Sigma, Oakville, Ontario, Canada) stock solution was made using double distilled  $\text{H}_2\text{O}$  and stored at 4°C. Quinpirole HCl (Tocris) stock solutions were prepared with double distilled  $\text{H}_2\text{O}$  then aliquoted and frozen. Fresh dopamine (3-hydroxytyramine-HCl, Sigma) stock was prepared each day with Na-metabisulfite (75  $\mu\text{M}$  final concentration, Fisher, Ottawa, Ontario, Canada).

## Statistical analysis

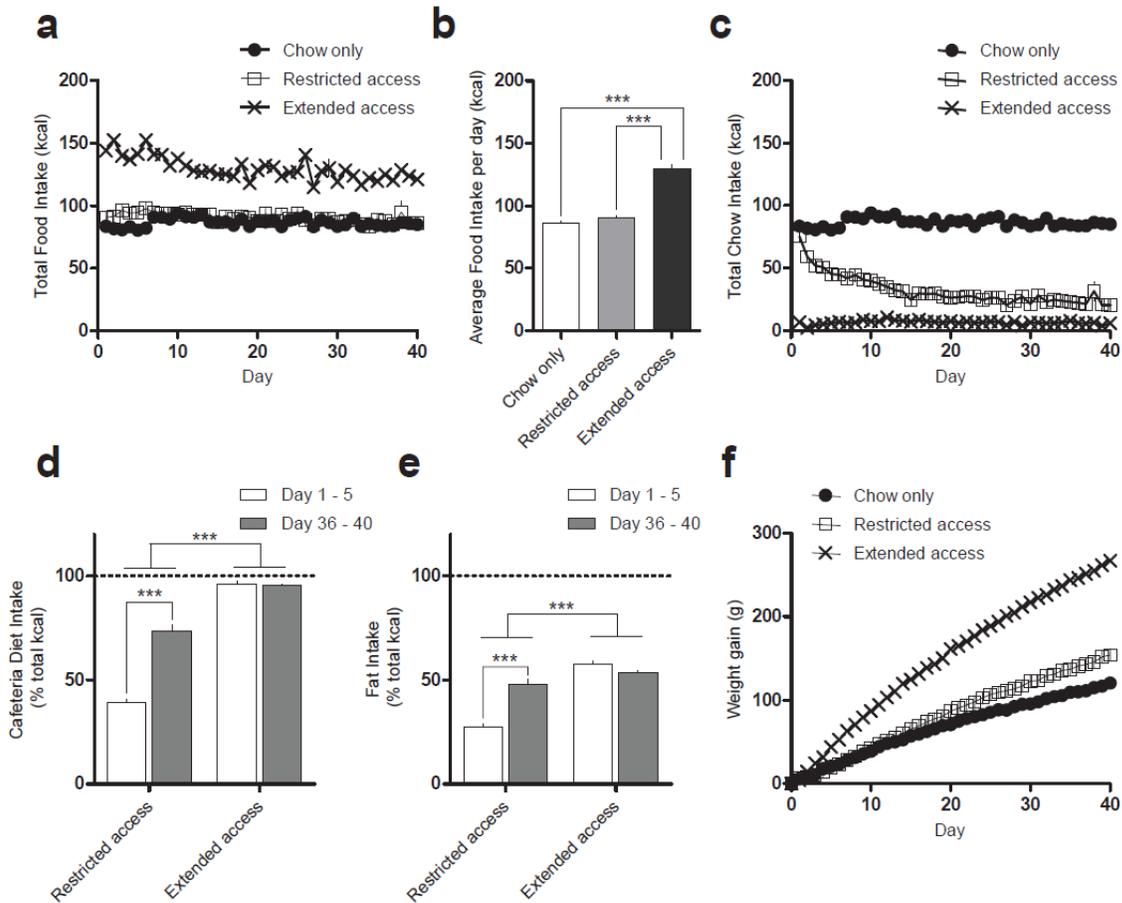
Data are expressed as mean  $\pm$  SEM. For weight gain, food consumption and plasma leptin or insulin concentrations “n” refers to the number of individual rats. For electrochemistry experiments “n” is expressed as the number of recordings/the number of individual rats

recordings came from, e.g.,  $n = 8/3$  represents 8 recordings from slices obtained from 3 separate rats. GraphPad Prism V. 5 was used to perform t-tests or 1- or 2-way analyses of variance (ANOVA) with Bonferroni's post-hoc test as appropriate.

## Results

### **Rats with extended access to a cafeteria diet gained more weight and consumed more calories than restricted access or chow only rats**

18 chow only, 19 restricted access and 20 extended access rats were fed and weighed daily for these studies. Their starting weights did not differ (chow only:  $323 \pm 9$  g vs restricted:  $324 \pm 9$  g vs extended:  $324 \pm 9$  g, 1-way ANOVA,  $F_{(2,54)}=0.009$ ,  $p > 0.05$ ). Rats with extended access to the cafeteria diet consumed more calories than restricted access or extended access rats (Figure 4.1 a,b). The average food intake per day was determined by finding the average daily calorie intake for each rat then comparing results for chow, extended and restricted access rats. The average calorie intake per day was significantly greater in rats with extended access to the cafeteria diet than for chow only or restricted access rats (chow only  $87 \pm 2$  kcal vs. restricted access  $91 \pm 2$  kcal vs. extended access  $130 \pm 3$  kcal, 1-way ANOVA,  $F_{(2,54)}= 123.2$ ,  $p < 0.001$ ). There was no difference in the total daily caloric intake between restricted access and chow only rats.



**Figure 4.1 Increased calorie consumption and weight gain in rats with extended access to a cafeteria diet.**

a) Total food consumption (kcal) over time for rats with access to chow alone (filled circles,  $n = 18$ ) or rats with restricted (open squares,  $n = 19$ ) or extended access (crosses,  $n = 20$ ) to a cafeteria diet. b) Average food intake per day (kcal) for chow only (open), restricted access (shaded) or extended access (filled dark grey) rats. c) Total chow consumption (kcal) over time for chow only (filled circles), restricted access (open squares) or extended access (crosses) rats. d) Calories from cafeteria diet as a per cent of total caloric intake in the first 5 days of measurement (open) and the last 5 days of measurement (shaded) for rats with restricted or extended access to a cafeteria diet. e) Calories from fat as a per cent of total calories in the first 5 days of measurement (open) and the last 5 days of measurement (shaded) for rats with restricted or extended access to a cafeteria diet. f) Weight gain over time in rats with access to chow alone (filled circles) or rats with restricted (open squares) or extended access (crosses) to a cafeteria diet. Some error bars are occluded by the symbols. \*\*\* =  $p < 0.001$ .

Extended access rats consumed few calories from chow (median through entire experiment 6 kCal, range 0 - 30.3 kCal Figure 4.1 c). To analyse whether the feeding behaviour of the rats changed over time, I compared the average daily caloric intake from the cafeteria diet in the first 5 days of the diet (days 1 - 5) to that of the last 5 days (days 36 – 40, Figure 4.1 d). A repeated measures 2-way ANOVA found a significant effect of diet indicating that the percentage of calories from the cafeteria diet was greater in extended access rats than restricted access rats (diet  $F_{(1,37)}=409.49$ ,  $p < 0.001$ , diet-time interaction  $F_{(1,37)}=105.55$ ,  $p < 0.001$ ). A Bonferroni's post-hoc test found no significant effect of time for the extended access rats. However, there was a significant increase in cafeteria diet consumption over time in the restricted access rats ( $P < 0.001$ ) which was concomitant with a decrease in chow intake (Figure 4.1 c). Taken together, these data indicate that over time restricted access rats escalate their cafeteria diet intake during the one hour of access each day, but regulate their total caloric intake by restricting chow consumption in the remaining 23 hours of the day.

Next, I determined if there were differences in macronutrient intake between the rats.

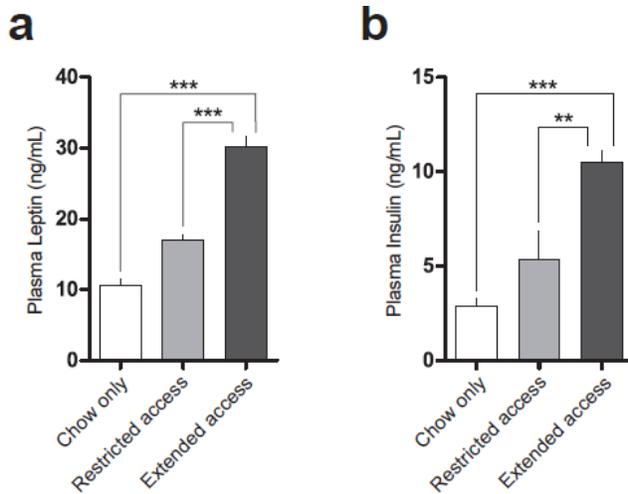
Extended access rats consumed a higher proportion of their calories from fat than restricted access or chow-fed rats (repeated measures 2-way ANOVA, diet  $F_{(1,36)}=65.99$ ,  $p < 0.001$ , Figure 4.1 e). There was an interaction between the % of calories obtained from fat and duration of diet (interaction  $F_{(1,36)}=79.12$ ,  $p < 0.001$ ). Post-hoc tests revealed that fat consumption did not change throughout the 40 days for the extended access rats (Day 1-5  $57 \pm 2\%$  vs. Day 36-40  $53 \pm 1\%$ ,  $p > 0.05$ ). However, the proportion of calories obtained from fat for restricted access rats was significantly increased at the end of the 40 day exposure (Day 1-5  $27 \pm 2\%$  vs. Day 36-40  $50 \pm 2\%$ ,  $p < 0.001$ ). Extended access rats also consumed a lower proportion of their diet from

protein or carbohydrates than restricted access rats (average % kcal from protein days 1 - 40, restricted  $18 \pm 0.5$  % vs. extended  $11 \pm 0.2$ %, t-test  $t_{(36)}=12.19$ ,  $p < 0.001$ ; average % kcal from carbohydrates days 1 - 40, restricted  $48 \pm 2$ % vs. extended  $38 \pm 1$  %, t-test  $t_{(36)}=4.5$ ,  $p < 0.001$ ). For comparison, the chow only diet provided 12% of calories from fat, 29 % of calories from protein and 59% of calories from carbohydrates (Table 4.1).

Rats with extended access to the cafeteria diet gained more weight than restricted access or chow-fed rats (Figure 4.1 f). At day 40 extended access rats were heavier than chow only and restricted access rats (chow only  $443 \pm 9$  g vs. restricted  $478 \pm 9$  g vs. extended  $591 \pm 13$  g, 1-way ANOVA,  $F_{(2,54)}=55.23$ , chow or restricted vs. extended  $p < 0.001$ ). There was no significant difference for weight at day 40 between chow and restricted rats ( $p > 0.05$ ).

Rats with extended access to the cafeteria diet also had significantly elevated leptin and insulin levels compared with restricted access or chow-fed rats (Figure 4.2 a,b). At the end of the 40 day access the average plasma leptin concentration in extended access rats was  $30 \pm 1$  ng/mL ( $n = 8$ , 1-way ANOVA,  $F_{(2,19)}=85.36$ ,  $p < 0.0001$ , Bonferroni's post-hoc test vs. chow  $11 \pm 1$  ng/mL,  $n = 8$ ,  $p < 0.001$ , vs. restricted,  $n = 6$ ,  $17 \pm 0.7$  ng/mL,  $p < 0.001$ , Figure 4.2 a). The average insulin concentration was  $10 \pm 0.7$  ng/mL ( $n = 8$ , 1-way ANOVA,  $F_{(2,18)}=27$ ,  $p < 0.0001$ , Bonferroni's post-hoc test vs. chow  $3 \pm 0.4$  ng/mL,  $n = 8$ ,  $p < 0.001$ , vs. restricted  $5 \pm 1.5$  ng/mL,  $n = 5$ ,  $p < 0.01$ , Figure 4.2 b). There were no differences in baseline measurements between groups for leptin or insulin concentrations (leptin: chow only  $5.9 \pm 1$  ng/mL,  $n = 8$ , vs. restricted  $5.6 \pm 0.4$  ng/mL,  $n = 6$ , vs. extended  $5.7 \pm 1$  ng/mL, 1-way ANOVA,  $F_{(2,19)}=0.02$ ,  $p = 0.98$ ; insulin: chow only

2.8 ± 0.7 ng/mL, n = 8, vs. restricted access 2.3 ± 0.5 ng/mL, n = 5, vs. extended access 2.4 ± 0.3 ng/mL, n = 8, 1-way ANOVA,  $F_{(2,18)}=0.25$ ,  $p = 0.78$ ).



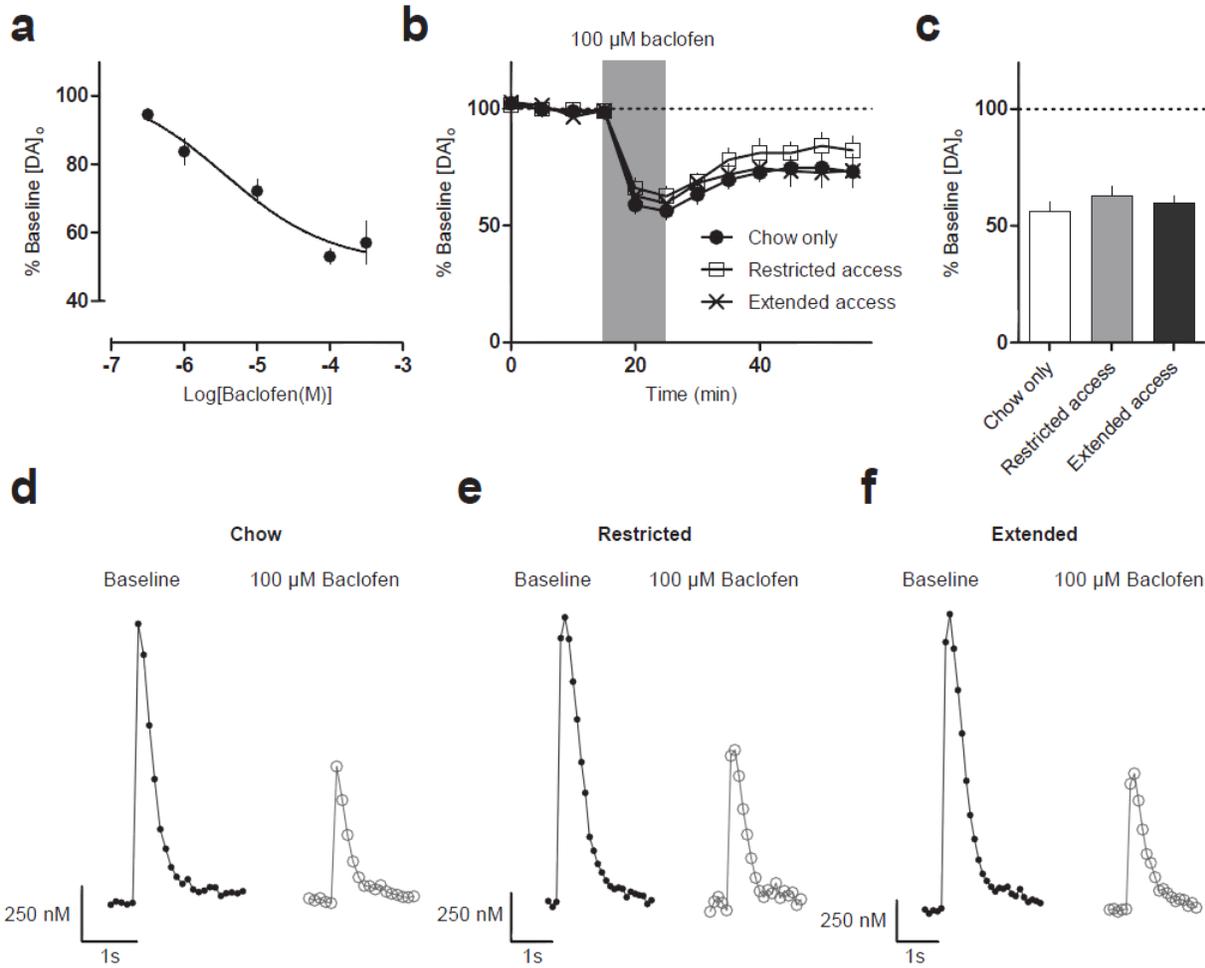
**Figure 4.2 Rats with extended access to a cafeteria diet have elevated plasma leptin and insulin**

a) Plasma leptin concentration in chow fed rats (open), or in rats after 40 days of restricted (shaded) or extended (filled dark grey) access to a cafeteria diet. b) Plasma insulin concentration in chow fed rats (open), or in rats after 40 days of restricted (shaded) or extended (filled dark grey) access to a cafeteria diet. \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

### **GABA<sub>B</sub> receptor modulation of dopamine release is unchanged by access to a cafeteria diet**

I used FCSV to assess whether restricted or extended access to the cafeteria diet altered GABA<sub>B</sub> receptor modulation of dopamine release in the NAc core. In Chapter 3, I demonstrated that baclofen inhibits dopamine release evoked by a single pulse electrical stimulus in the NAc core of C57BL6 mice. To test if baclofen similarly inhibits dopamine release in naïve adult rats, baclofen (300 nM - 300 μM) was applied to ventral striatal slices during single-pulse evoked dopamine release. Baclofen concentration-dependently inhibited evoked dopamine release in

the NAc core of control rats with an  $IC_{50}$  of  $3.6 \pm 0.8 \mu\text{M}$  (1 concentration per slice,  $0.3 \mu\text{M}$ ,  $n = 6/3$ ;  $1 \mu\text{M}$ ,  $n = 5/3$ ;  $10 \mu\text{M}$ ,  $n = 6/3$ ;  $100 \mu\text{M}$ ,  $n = 7/3$ ;  $300 \mu\text{M}$ ,  $n = 7/4$ , Figure 4.3 a) to a maximal inhibition of  $53 \pm 2 \%$  of baseline. To determine if access to a cafeteria diet alters baclofen-mediated inhibition of dopamine release, I applied a maximal concentration of baclofen to NAc core slices from either chow-fed, or rats with restricted or extended access to a cafeteria diet (Figure 4.3 b-f). Baclofen ( $100 \mu\text{M}$ , 10 mins) inhibited dopamine release in chow-fed rats to  $56 \pm 4 \%$  of baseline ( $n = 10/4$ ). However, restricted or extended access to a cafeteria diet did not alter baclofen-inhibition of evoked dopamine release. Baclofen inhibited evoked dopamine release to  $63 \pm 4 \%$  of baseline in restricted access rats ( $n = 8/3$ ) and to  $60 \pm 3 \%$  of baseline in extended access rats ( $n = 8/3$ , 1-way ANOVA,  $F_{(2,23)}=0.7$ ,  $p > 0.05$ ). Taken together, these results suggest that while baclofen concentration-dependently inhibits dopamine release in control rats, access to a cafeteria diet does not influence the efficacy of baclofen-inhibition of dopamine release.

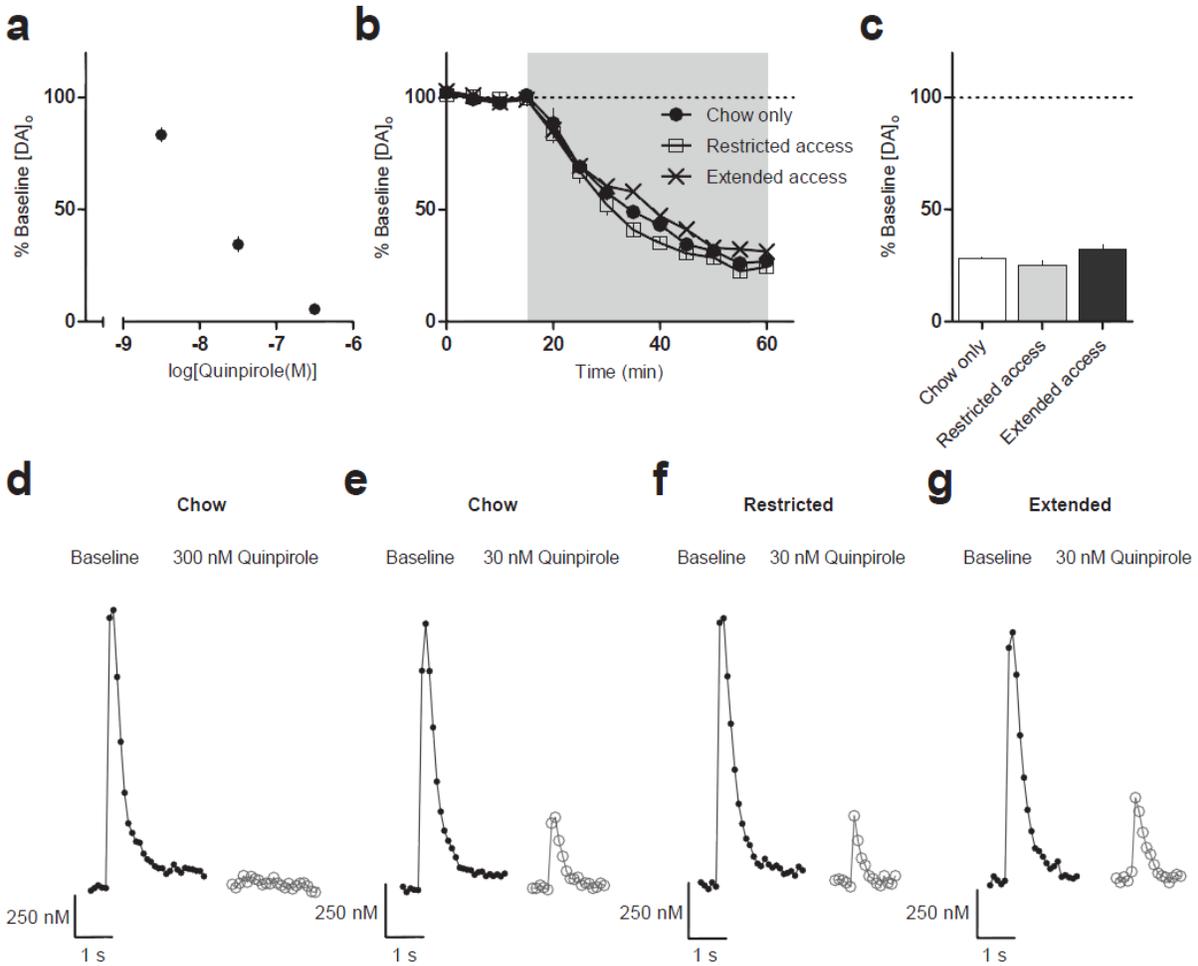


**Figure 4.3 GABA<sub>B</sub> modulation of dopamine release in the NAC core is unaltered by cafeteria diet access**

a) Concentration response curve for baclofen inhibition of dopamine release evoked with a single pulse electrical stimulus in the NAC core of chow control rats. b) The time course of bath application of baclofen (100 μM) on dopamine release in brain slices from chow only (filled circles), restricted access (open squares) or extended access (crosses) rats. c) Bar graph demonstrating the maximal effect of baclofen (100 μM, 10 min) on dopamine release in chow only (open), restricted access (shaded) or extended access (filled) rats. d-f) Example concentration vs. time plots for baclofen (100 μM) effect on dopamine release in brain slices from chow only (d), restricted access (e) or extended access (f) rats.

## **D2 receptor modulation of dopamine release is unchanged by access to a cafeteria diet**

To test if D2-modulation of dopamine release is modified by access to a cafeteria diet, I used fast scan cyclic voltammetry to measure the effect of the D2 receptor agonist quinpirole on single pulse-evoked dopamine release in the NAc core. Quinpirole concentration-dependently inhibited dopamine release in control rats (1 concentration per slice, 3 nM, n = 3/3; 30 nM, n = 5/4; 300 nM, n = 4/3, Figure 4.4 a). Because 300 nM quinpirole inhibited dopamine release to below detectable levels ( $6 \pm 2\%$  of baseline, n = 4/3, Figure 4.4 a,d), I used 30 nM quinpirole to test if access to a cafeteria diet modulates quinpirole-mediated inhibition. In chow-fed rats, 30 nM quinpirole decreased dopamine release to  $28 \pm 1\%$  of baseline (n = 5/4, Figure 4.4 b,c,e). This was not significantly different from quinpirole-mediated inhibition of dopamine release in NAc core of rats with restricted ( $25 \pm 2\%$  of baseline, n = 5/3, Figure 4.4 c,f) or extended access ( $32 \pm 2\%$  of baseline, n = 5/4, Figure 4.4 c,g) to the cafeteria diet. Taken together, modulation of dopamine concentration by quinpirole was not altered by access to a cafeteria diet or obesity.

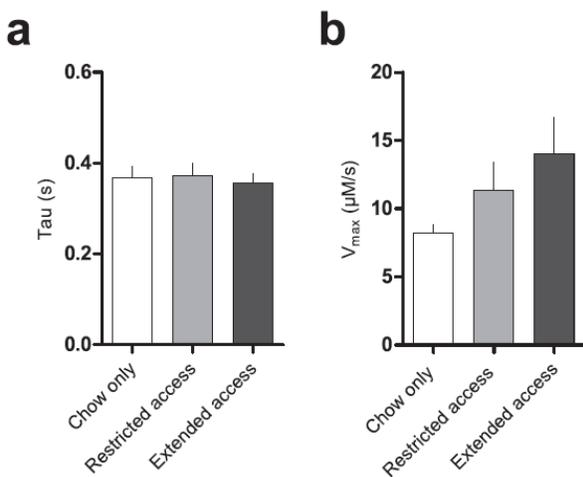


**Figure 4.4 D2 modulation of dopamine release in the NAc core is unaltered by cafeteria diet access**

a) The effect of 3 concentrations of quinpirole on inhibition of dopamine release evoked with a single pulse electrical stimulus in the NAc core of chow control rats. b) The time course of bath application of quinpirole (30 nM) on dopamine release in brain slices from chow only (filled circles), restricted access (open squares) or extended access (crosses) rats. c) Bar graph demonstrating the effect of a submaximal concentration of quinpirole (30 nM, 30-45 min) on dopamine release in chow only (open), restricted access (shaded) or extended access (filled) rats. d) Example concentration vs. time plots for quinpirole (300 nM) effect on dopamine release in brain slices from chow only control rats. e-g) Example concentration vs. time plots for quinpirole (30 nM) effect on dopamine release in brain slices from chow only (e), restricted access (f) or extended access (g) rats.

## Dopamine re-uptake is unchanged by access to a cafeteria diet

To test if extended or restricted access to a cafeteria diet altered dopamine reuptake I measured the tau and  $V_{max}$  of the clearance of electrically evoked dopamine. A 1-way ANOVA found no significant difference in tau ( $F_{(2,26)}=0.090$ ,  $p = 0.91$ , Figure 4.5 a) or  $V_{max}$  ( $F_{(2,26)}=1.925$ ,  $p = 0.17$ , Figure 4.5 b) between chow-fed rats ( $n = 9/5$ ) and rats with restricted ( $n = 10/6$ ) or extended ( $n = 10/5$ ) access to the cafeteria diet.



**Figure 4.5 Dopamine reuptake in the NAc core is unaltered by access to a cafeteria diet**

a) Time constant of dopamine reuptake for chow-fed rats (open) or rats with restricted (shaded) or extended (filled) access to a cafeteria diet. b) Maximal rate of dopamine reuptake for chow-fed rats (open) or rats with restricted (shaded) or extended (filled) access to a cafeteria diet.

## Discussion

Here I examined how 3 mechanisms regulating the extracellular dopamine concentration were modulated by diet-induced obesity. I found that dopamine reuptake or D2 or GABA<sub>B</sub> receptor mediated inhibition of terminal dopamine release in the NAc core was not altered after

extended or restricted access to a cafeteria diet. Importantly, rats with extended access to the cafeteria diet were heavier than their chow fed or restricted access counterparts by day 40 of being on the diet and had significantly higher insulin and leptin levels, meeting criteria for obesity (Rothwell and Stock, 1981). Similar to other studies employing a cafeteria diet (Rolls et al., 1980; Johnson and Kenny, 2010; van de Giessen et al., 2012) induction of obesity was faster than what would be expected in rats given extended access rats to a single high fat chow (Cone et al., 2013). The rapid weight gain was likely due to the variety of foods available minimising the effect of sensory specific satiety, where rats can become satiated to a specific food type (Johnson and Kenny, 2010; van de Giessen et al., 2012; la Fleur et al., 2014).

Animals with restricted access to the cafeteria diet exhibited a binge-pattern of feeding as their cafeteria diet consumption, consumed within a one hour period each day, escalates over the 40 days of cafeteria diet access. Interestingly, as the experiment progressed these rats turned down chow intake to increase their consumption of the cafeteria diet. While this did not equate to an increase in total daily caloric intake, it did represent binge pattern of feeding where rats consumed the majority of their daily caloric intake during the 1 hour cafeteria diet access.

### **GABA<sub>B</sub> receptors on dopamine neuron terminals are resistant to diet-induced alterations**

Activation of GABA<sub>B</sub> receptors on dopamine neuron terminals inhibits dopamine release in the NAc core of mice (Chapter 3) (Pitman et al., 2014). Similar to the results in naïve C57BL6 mice, baclofen concentration-dependently inhibited dopamine release in rats. I found that restricted or extended access to a cafeteria diet did not modulate baclofen-mediated inhibition of dopamine release, suggesting that obese or binge-feeding rats do not have altered GABA<sub>B</sub>-

mediated suppression of dopamine release in the NAc core. To date, no previous studies have explored changes in GABA<sub>B</sub> receptor expression or function after access to a high fat palatable diet. Continued baclofen-induced inhibition of dopamine in obese animals is consistent with results reporting no changes in GABA<sub>B</sub> modulation of dopamine release after sensitising cocaine treatment or withdrawal (Pitman et al., 2014). Previous work has demonstrated reduced extracellular dopamine with obesity (Geiger et al., 2009). Future studies should determine if extracellular GABA levels in the NAc are altered by diet induced obesity. Increased GABA acting at GABA<sub>B</sub> receptors on dopamine neuron terminals that do not desensitise or down regulate could cause a chronic inhibition of dopamine release and contribute to the reduced extracellular dopamine observed after diet-induced obesity (Geiger et al., 2009).

The GABA<sub>B</sub> receptor agonist baclofen consistently reduces responding for and the rewarding effects of drugs of abuse in rodents (Filip et al., 2015) but has inconsistent results for responses related to feeding. There have been reports that baclofen can both decrease (Zarrindast et al., 1989; Buda-Levin et al., 2005) and increase (Ebenezer, 1990; Bains and Ebenezer, 2013) food consumption. Previous literature has suggested that baclofen may be more efficacious in reducing the intake of high fat food when access to the high fat food has been given in an intermittent fashion over a period of time resulting in binge like-consumption (Buda-Levin et al., 2005; Berner et al., 2009). Our results demonstrating baclofen-inhibition of dopamine release is resistant to pathophysiological changes support GABA<sub>B</sub> agonists as potentially efficacious agents for treating binge-eating disorders (Broft et al., 2007; Corwin et al., 2012).

## **D2 autoreceptor function in the NAc core is intact after diet induced obesity**

In contrast to the lack of studies examining diet-induced changes in GABA<sub>B</sub> receptor expression or function, many studies have examined diet-induced changes in D2 receptor expression.

However, to date, no one has assessed whether diet-induced obesity can alter presynaptic D2 receptor function. I found that regulation of dopamine release in the NAc core by D2 receptors was unchanged after restricted or extended access to a cafeteria diet, suggesting feedback inhibition of dopamine release is intact in the NAc core after diet induced obesity. This also suggests that the previously reported changes in D2 receptor expression in the NAc are not likely due to changes in D2 receptors expressed on dopamine neuron terminals in the NAc core (Huang et al., 2005; South and Huang, 2008; Sharma and Fulton, 2013; van de Giessen et al., 2013).

Several reasons may account for the discrepancy between our results and obesity-induced changes in D2 receptor expression observed by others. While I observed no effect of D2 receptor modulation of dopamine release in the NAc core, it is feasible that diet induced obesity can modulate D2 receptor expression in other striatal regions. Our studies focused on the NAc core as it is important for mediating cue induced food-seeking behaviours (Bossert et al., 2007; Floresco et al., 2008; Chaudhri et al., 2010; Ambroggi et al., 2011). Other studies that have found obesity-associated decreases in D2 receptor expression either examined changes in the dorsal striatum (Colantuoni et al., 2001; van de Giessen et al., 2012) or did not specify the specific striatal region studied (Hamdi et al., 1992; Johnson and Kenny, 2010; Narayanaswami et al., 2013). In addition, results of research examining obesity induced changes in D2 receptor expression in the NAc have been inconsistent with both decreases (Hajnal et al., 2008; van de

Giessen et al., 2013), and increases (Huang et al., 2005; South and Huang, 2008; Sharma and Fulton, 2013) in D2 receptor expression being reported. Future studies should be directed at measuring D2 mediated regulation of dopamine release in other striatal regions such as the dorsal lateral striatum or NAc shell.

While I observed no alteration in D2 regulation of dopamine release, it is possible that postsynaptic D2 receptor expression or function could be modified with diet induced obesity. Others have used techniques such as receptor autoradiography (Hajnal et al., 2008), western blots (Johnson and Kenny, 2010) or radioligand binding (Narayanaswami et al., 2013) to quantify D2 receptor expression, which do not discriminate between presynaptic or postsynaptic expression. Therefore, D2 receptor expression at presynaptic terminals may be intact, whereas changes in D2 receptor expression on indirect pathway MSNs may account for overall changes. Future studies should be directed at testing if restricted or extended access to a cafeteria diet reduces D2 receptor expression on MSNs in the NAc. Importantly, the inhibitory D2 receptors are also located on cholinergic interneurons in the dorsal and ventral striatum (Dawson et al., 1988; Ding et al., 2010). Because ACh action at nACh receptors on dopamine neuron terminals increases dopamine release (Zhou et al., 2001; Zoli et al., 2002; Salminen et al., 2004; Zhang and Sulzer, 2004), changes in D2 receptors on cholinergic interneurons may result in altered ACh release upon quinpirole application and, thus, could occlude any effects of altered D2 receptors on dopamine neuron terminals. Further experimentation is required to test the contribution of diet induced obesity to D2 modulation of cholinergic regulation of dopamine release.

## **Dopamine reuptake was not altered by access to a cafeteria diet**

I found no changes in dopamine reuptake after access to a cafeteria diet suggesting that the rapid clearance of extracellular dopamine in the NAc core is unchanged by diet induced obesity. Others have reported (South and Huang, 2008) decreased dopamine transporter binding in the NAc shell after 20 days access to a high fat diet using quantitative autoradiography. However dopamine transporter binding in the NAc core was not reported. Furthermore, other studies demonstrating diet induced changes in dopamine reuptake did not specify the striatal region assessed (Narayanaswami et al., 2013) or included the dorsal-medial striatum and NAc core together in the assessment of transporter function (Cone et al., 2013). Thus, it is possible that dopamine transporter expression is altered in other striatal regions but not in the NAc core. Another difference between the current study and the studies reporting altered dopamine reuptake is that the other studies only offered rats a single high fat food (South and Huang, 2008; Speed et al., 2011; Cone et al., 2013; Narayanaswami et al., 2013). In order to determine whether the availability of choice in a diet can affect alterations in dopamine transporter expression or function, dopamine reuptake after extended access to a free choice cafeteria diet should be compared to dopamine reuptake after access to a single nutritionally matched food.

## **Summary and future directions**

Our results suggest that dopamine reuptake and presynaptic regulation of dopamine release by D2 and GABA<sub>B</sub> receptors in the NAc core are unchanged by restricted or extended access to a palatable cafeteria diet. Other neurotransmitters such as ACh (Zhou et al., 2001; Zoli et al., 2002; Salminen et al., 2004; Zhang and Sulzer, 2004) and glutamate (Zhang and Sulzer, 2003)

also have direct effects on dopamine release. Future studies on these neurotransmitters will give a clearer picture of how dopamine release is altered by diet-induced obesity.

## **Chapter 5 General discussion and conclusions**

This thesis investigates actions of known or putative GABA<sub>B</sub> receptor agonists at heterologously or natively expressed GABA<sub>B</sub> receptors in isolated cells or natively expressed GABA<sub>B</sub> receptors in brain slices containing the NAc core. In Chapter 2, I found that the putative GABA<sub>B</sub> receptor agonist, R-isovaline, did not activate GABA<sub>B</sub>-mediated K<sup>+</sup> currents in isolated cells. In Chapter 3, I demonstrated that the GABA<sub>B</sub> receptor agonist baclofen reduced evoked dopamine release in the NAc core, an effect due to a direct action of GABA<sub>B</sub> receptors located on dopamine neuron terminals. I also demonstrated that the ability of baclofen to inhibit dopamine release was not altered by a sensitising cocaine treatment. Finally, in Chapter 4 I found that GABA<sub>B</sub> or D2 receptor modulation of dopamine release was unaltered by extended or restricted access to a palatable cafeteria diet. Below I highlight the key findings of this work and discuss their implications, limitations and potential future directions.

### **R-isovaline does not act as a GABA<sub>B</sub> receptor agonist**

My data failed to support the hypothesis that R-isovaline is a GABA<sub>B</sub> receptor agonist. I used a heterologous expression system to explore the effects of R-isovaline in comparison with known agonists of the GABA<sub>B</sub> receptor. Agonist action at the GABA<sub>B</sub> receptor was defined as the ability to evoke an inwardly rectifying K<sup>+</sup> conductance. The benefits of this technique are that it allows measurement of direct effects of ligands on GABA<sub>B</sub> receptors in isolation from factors that could potentially produce indirect effects, such as other receptors, network activity and mediation by glial cells (Chang et al., 2007; Angulo et al., 2008; Boyer et al., 2009). However, this isolated system also carries some limitations as expression of GABA<sub>B</sub> receptor subunits in heterologous expression systems does not fully recapitulate the native GABA<sub>B</sub> receptor, which

exists in a large signalling complex alongside the effector and auxiliary subunits and other regulatory proteins (Fowler et al., 2007; Schwenk et al., 2010; Laviv et al., 2011). Despite this, all other current GABA<sub>B</sub> ligands have shown activity at heterologously expressed GABA<sub>B</sub> receptors, albeit with lower affinity than what would be expected from native receptor preparations (Bräuner-Osborne and Krogsgaard-Larsen, 1999; Lingenhoebl et al., 1999; Urwyler et al., 2005; Lehmann et al., 2009; Berecki et al., 2014). As R-isovaline's effects in thalamic slices were deemed to be atypical I also measured R-isovaline actions at natively expressed GABA<sub>B</sub> receptors. I chose cultured hippocampal neurons for their well characterised and readily measurable responses to GABA<sub>B</sub> receptor agonists (Sodickson and Bean, 1996).

I found that extracellular or intracellular application of R-isovaline had no effect on membrane currents in AtT-20 cells heterologously expressing GABA<sub>B</sub> receptors or in isolated hippocampal neurons that endogenously express GABA<sub>B</sub> receptors, while extracellular application of baclofen or GABA consistently evoked inward currents. R-isovaline did not interfere or alter the ability of baclofen or GABA to induce inwards currents suggesting that R-isovaline does not occlude the binding, receptor activation or downstream consequences of these ligands. Nor could I find evidence that R-isovaline allosterically modulates the GABA<sub>B</sub> receptor.

There are numerous possibilities as to why R-isovaline did not activate GABA<sub>B</sub> receptor coupled K<sup>+</sup> conductances in isolated cells, but required GABA<sub>B</sub> receptors to initiate an effect in thalamocortical neurons (Cooke et al., 2012). GABA<sub>B</sub> subunits have been shown to associate with Ca<sup>2+</sup> sensing receptors and M2 muscarinic ACh receptors (Chang et al., 2007; Cheng et al., 2007; Boyer et al., 2009). Thus, it is possible that R-isovaline may require association of

different receptor populations in order to initiate its effects. Recently, R-isovaline was reported to induce antiallodynia via  $G_{\alpha i/o}$ -coupled group 2 mGluRs, an effect that was blocked by a group 2 mGluR antagonist (Asseri et al., 2015). However, the group 2 mGluR antagonist did not modify baclofen-induced antiallodynia and a  $GABA_B$  receptor antagonist did not alter antiallodynia mediated by a group 2 mGluR agonist, suggesting a lack of cross-talk between the 2 receptor types (Asseri et al., 2015). Future studies should be directed at determining the possibility of R-isovaline acting as a direct agonist at group 2 mGluRs, and whether isovaline mediated  $K^+$  conductances in thalamocortical neurons require co-activation of group 2 mGluRs and  $GABA_B$  receptors.

Another possibility for a lack of effect of R-isovaline in the experiments detailed in Chapter 2 is that R-isovaline could act as a biased agonist of the  $GABA_B$  receptor and thus only initiate specific signalling cascades. I measured  $GABA_B$  mediated activation of GIRK channels because this is a well characterised response to  $GABA_B$  receptor activation and is readily measurable using whole cell patch clamp electrophysiology. Activation of the  $GABA_B$  receptor results in exchange of GDP for GTP on the associated G-protein which enables dissociation of the G-protein into  $G_\alpha$  and  $G_{\beta\gamma}$ . The free  $G_{\beta\gamma}$  inhibits VGCCs and activated GIRK channels in a membrane delimited manner while the dissociated  $G_\alpha$  subunit inhibits production of cAMP (Hill, 1985; Xu and Wojcik, 1986; Mintz and Bean, 1993; Kofuji et al., 1995; Sadjja et al., 2003; David et al., 2006; Rubinstein et al., 2007; Doupnik, 2008; Ciruela et al., 2010). To determine if R-isovaline is able to activate G-proteins associated with  $GABA_B$  receptors a radiolabelled GTP- $\gamma$ -S binding assay could be employed (Harrison and Traynor, 2003; Willars and Challiss, 2004). Future studies could also examine the effects of R-isovaline on inhibition of forskolin stimulated cAMP

production, inhibition of VGCCs, or on non-canonical effects of GABA<sub>B</sub> receptor activation such as activation of extracellular signal-regulated protein kinases (Tu et al., 2007). Interestingly, it has recently been demonstrated that unlike baclofen-mediated analgesia, the GABA<sub>B</sub>-mediated analgesic effect of the  $\alpha$ -conotoxin, Vc1.1, is facilitated via a novel mechanism involving inhibition of N-type VGCCs by the GABA<sub>B1</sub> subunit C-terminal domain (Huynh et al., 2015). It is possible that antiallodynia produced by R-isovaline could involve this novel GABA<sub>B</sub> mediated response. Future studies should focus on the mechanism of action of R-isovaline in tissues where it has previously been shown that the GABA<sub>B</sub> receptor is involved in mediating R-isovaline's response (Cooke et al., 2012; Whitehead et al., 2012).

While I was unable to elucidate the mechanism of action of R-isovaline, the unusual mechanism of action may prove advantageous. GABA<sub>B</sub> receptor agonists can cause adverse CNS side effects (Sawa and Paty, 1979; Nakagawa et al., 1996; Quéva et al., 2003; van Nieuwenhuijzen and McGregor, 2009; Whitehead et al., 2012). The novel mechanism of R-isovaline may allow for positive therapeutic effects without the side effects typical of GABA<sub>B</sub> receptor agonists.

Whether R-isovaline is beneficial in other disorders involving GABA<sub>B</sub> receptors warrants further investigation.

### **GABA<sub>B</sub> receptor activation decreases dopamine release in the NAc core of both mice and rats**

Using fast scan cyclic voltammetry I demonstrated that GABA<sub>B</sub> receptors reduce electrically evoked dopamine release in the NAc core of both mice (Chapter 3) and rats (Chapter 4). The IC<sub>50</sub> for baclofen inhibition of dopamine release was similar (3.7  $\mu$ M in mice and 3.6  $\mu$ M in rats). Interestingly baclofen appeared to have a greater maximal effect on inhibiting dopamine

release in rats than in mice. 100  $\mu$ M baclofen inhibited dopamine release to an average of  $53 \pm 2$  % of baseline in rats while in mice 100  $\mu$ M baclofen inhibited dopamine release to an average of  $76 \pm 4$  % of baseline. These results suggest that GABA<sub>B</sub> receptor number at dopamine terminals may be greater in rats than mice. Notably, experiments in rats were undertaken in their dark cycle while experiments in mice were undertaken in the light cycle, thus, it is possible receptor expression may be diurnally regulated, similar to  $\mu$ -opioid receptors (Jabourian et al., 2005).

Signals recorded using fast scan cyclic voltammetry were attributed to dopamine if the voltammogram (plot of current versus voltage) demonstrated a characteristic shape. While the shape of the voltammogram can distinguish between dopamine and serotonin, or dopamine and dopamine metabolites such as 3,4-dihydroxyphenylacetic acid (Baur et al., 1988; Rebec et al., 1997), it is not able to distinguish between dopamine and noradrenaline (Baur et al., 1988; Heien et al., 2003). Noradrenaline is released into the NAc, however, the majority of noradrenergic input into the NAc is into the caudal region of the NAc shell (Berridge et al., 1997; Park et al., 2010). The expression of the noradrenaline synthetic enzyme, dopamine  $\beta$ -hydroxylase, in the NAc core is very low and the tissue content of noradrenaline within the core of the NAc is approximately 2% of the dopamine concentration (Garris et al., 1993; Berridge et al., 1997). In addition, the carbon fibre electrodes used for detecting catecholamine oxidation and reduction are more sensitive to dopamine than they are for noradrenaline or 3,4-dihydroxyphenylacetic acid (el Ganouni et al., 1987; Heien et al., 2003). For these reasons it is likely that the contribution of noradrenaline or dopamine metabolites to the recordings obtained from the NAc core in these experiments is negligible.

Baclofen-mediated inhibition of dopamine release occurred by a direct action on dopaminergic nerve terminals. The effect of baclofen was reduced by stimulations mimicking burst firing of dopamine neurons. In addition baclofen increased the paired pulse ratio of dopamine release. Dopamine D2 receptors also inhibit dopamine release by a presynaptic mechanism and increase the paired pulse ratio of dopamine release (Schmitz et al., 2002; Zhang and Sulzer, 2004). D2 and GABA<sub>B</sub> receptors both couple to the pertussis toxin sensitive G<sub>i/o</sub> family of G-proteins, thus, it is conceivable that D2 and GABA<sub>B</sub> receptors could inhibit dopamine release by a similar mechanism of action (Hill, 1985; Xu and Wojcik, 1986; Dal Toso et al., 1989; Morishita et al., 1990). In rats the action of quinpirole was much slower than that of baclofen (10 minutes vs. ~30 minutes) which could indicate different mechanisms of actions but could also be due to different chemical and binding properties of the agonists. It is unknown whether D2 receptors inhibit dopamine release by a direct coupling to VGCCs or whether they activate an axonal K<sup>+</sup> conductance to shunt action potentials arriving at the nerve terminal (Cardozo and Bean, 1995; Congar et al., 2002; Fulton et al., 2011; Martel et al., 2011). Further studies are needed in order to determine the downstream effects of activation of GABA<sub>B</sub> receptors on dopaminergic nerve terminals.

**GABA<sub>B</sub> receptors on dopamine neuron terminals are resistant to changes induced by sensitising cocaine treatment or access to a palatable cafeteria diet.**

I found that a sensitising cocaine treatment, or extended or restricted access to a cafeteria diet did not alter the inhibition of dopamine release induced by a maximal concentration of baclofen. In addition to dopamine neuron terminals, GABA<sub>B</sub> receptors are also expressed on GABA and glutamate neuron terminals in the NAc (Uchimura and North, 1991; Lacey et al.,

2005; Molnár et al., 2009). It is possible that cocaine treatment or diet-induced obesity could modulate GABA<sub>B</sub> regulation of glutamatergic or GABAergic terminals in the NAc. To explore whether these GABA<sub>B</sub> receptors are altered by sensitising cocaine treatment or by access to a cafeteria diet, patch clamp experiments looking at GABA<sub>B</sub> modulation of evoked and spontaneous GABA or glutamate release could be undertaken. Accordingly, GABA<sub>B</sub> receptors in the VTA and mPFC have been shown to be modulated by drugs of abuse such as cocaine or methamphetamine (Padgett et al., 2012; Hearing et al., 2013). Thus, future experiments aimed at examining changes in GABA<sub>B</sub> receptor function after access to a high fat diet should examine changes in these areas.

I looked at the effects of 2 access regimens to the palatable cafeteria diet, extended access and restricted access. Restricted access to the cafeteria diet was to test whether any observed changes were due to obesity or due to nutrient composition of the cafeteria diet. Restricted access to the cafeteria diet resulted in binge-like consumption of food. It has previously been shown that rodents subjected to restricted access to food such that their feeding occurs in binges show increased motivation for cocaine (Puhl et al., 2011) and enhanced locomotor responses to amphetamine (Avena and Hoebel, 2003; Gosnell, 2005). This indicates that binge-like feeding may be associated with neuroadaptations in the mesolimbic system (Cottone et al., 2008). However, I did not observe any alterations in dopamine reuptake or inhibition of dopamine release by D2 or GABA<sub>B</sub> receptor agonists. To control for exposure to the cafeteria diet without the effect of increased caloric consumption and weight gain, an alternative group could be given 24 hour access to a controlled amount of cafeteria diet that is calorie matched to the daily calories consumed by chow-fed rats but the nutrient composition is matched to

that consumed by the extended access rats. However, this was not feasible for the current study as the average daily caloric intake or macronutrient composition of consumed food for the rats fed standard chow or given extended access to the cafeteria diet had not previously determined.

## **Summary**

In summary, I have shown the effects of known and putative ligands for the GABA<sub>B</sub> receptor in heterologous, physiological and pathophysiological circumstances. I demonstrated that the putative GABA<sub>B</sub> agonist, R-isovaline, does not activate GABA<sub>B</sub>-coupled K<sup>+</sup> currents in isolated cells. I also demonstrated that baclofen inhibits dopamine release in the NAc core of both mice and rats and revealed that GABA<sub>B</sub> receptors on dopamine neuron terminals are resistant to alterations by cocaine or diet.

## References

- Adelfinger, L., Turecek, R., Ivankova, K., Jensen, A.A., Moss, S.J., Gassmann, M., et al. (2014). GABAB receptor phosphorylation regulates KCTD12-induced K<sup>+</sup> current desensitization. *Biochem. Pharmacol.* *91*: 369–79.
- Adler, A., Katabi, S., Finkes, I., Prut, Y., and Bergman, H. (2013). Different correlation patterns of cholinergic and GABAergic interneurons with striatal projection neurons. *Front. Syst. Neurosci.* *7*: 47.
- Albanese, A., and Minciacchi, D. (1983). Organization of the ascending projections from the ventral tegmental area: a multiple fluorescent retrograde tracer study in the rat. *J. Comp. Neurol.* *216*: 406–20.
- Ambroggi, F., Ghazizadeh, A., Nicola, S.M., and Fields, H.L. (2011). Roles of nucleus accumbens core and shell in incentive-cue responding and behavioral inhibition. *J. Neurosci.* *31*: 6820–6830.
- Anderson, J.J., Kuo, S., Chase, T.N., and Engber, T.M. (1993). GABA<sub>A</sub> and GABA<sub>B</sub> receptors differentially regulate striatal acetylcholine release in vivo. *Neurosci. Lett.* *160*: 126–30.
- Angulo, M.C., Meur, K. L, Kozlov, A.S., Charpak, S., and Audinat, E. (2008). GABA, a forgotten gliotransmitter. *Prog. Neurobiol.* *86*: 297–303.
- Anstrom, K.K., Cromwell, H.C., Markowski, T., and Woodward, D.J. (2003). Effect of baclofen on alcohol and sucrose self-administration in rats. *Alcohol. Clin. Exp. Res.* *27*: 900–8.
- Arora, D., Hearing, M., Haluk, D.M., Mirkovic, K., Fajardo-Serrano, A., Wessendorf, M.W., et al. (2011). Acute cocaine exposure weakens GABA(B) receptor-dependent G-protein-gated inwardly rectifying K<sup>+</sup> signaling in dopamine neurons of the ventral tegmental area. *J. Neurosci.* *31*: 12251–7.
- Asseri, K.A., Puil, E., Schwarz, S.K.W., and MacLeod, B.A. (2015). Group II metabotropic glutamate receptor antagonism prevents the antiallostatic effects of R-isovaline. *Neuroscience* *293*: 151–156.
- Avena, N.M., and Hoebel, B.G. (2003). A diet promoting sugar dependency causes behavioral cross-sensitization to a low dose of amphetamine. *Neuroscience* *122*: 17–20.
- Avshalumov, M. V., Chen, B.T., Marshall, S.P., Peña, D.M., and Rice, M.E. (2003). Glutamate-dependent inhibition of dopamine release in striatum is mediated by a new diffusible messenger, H<sub>2</sub>O<sub>2</sub>. *J. Neurosci.* *23*: 2744–50.

Avshalumov, M. V., and Rice, M.E. (2003). Activation of ATP-sensitive K<sup>+</sup> (K(ATP)) channels by H<sub>2</sub>O<sub>2</sub> underlies glutamate-dependent inhibition of striatal dopamine release. *Proc. Natl. Acad. Sci. U. S. A.* *100*: 11729–34.

Bains, R.S., and Ebenezzer, I.S. (2013). Effects of the GABA(B) receptor agonist baclofen administered orally on normal food intake and intraperitoneally on fat intake in non-deprived rats. *Eur. J. Pharmacol.* *698*: 267–71.

Balasubramanian, S., Fam, S.R., and Hall, R.A. (2007). GABAB receptor association with the PDZ scaffold Mupp1 alters receptor stability and function. *J. Biol. Chem.* *282*: 4162–71.

Bamford, N.S., Robinson, S., Palmiter, R.D., Joyce, J.A., Moore, C., and Meshul, C.K. (2004). Dopamine modulates release from corticostriatal terminals. *J. Neurosci.* *24*: 9541–52.

Barral, J., Toro, S., Galarraga, E., and Bargas, J. (2000). GABAergic presynaptic inhibition of rat neostriatal afferents is mediated by Q-type Ca<sup>2+</sup> channels. *Neurosci. Lett.* *283*: 33–36.

Baur, J.E., Kristensen, E.W., May, L.J., Wiedemann, D.J., and Wightman, R.M. (1988). Fast-scan voltammetry of biogenic amines. *Anal. Chem.* *60*: 1268–72.

Bassareo, V., and Di Chiara, G. (1999). Modulation of feeding-induced activation of mesolimbic dopamine transmission by appetitive stimuli and its relation to motivational state. *Eur. J. Neurosci.* *11*: 4389–4397.

Bean, B.P. (1989). Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence. *Nature* *340*: 153–156.

Bechtholt, A.J., and Cunningham, C.L. (2005). Ethanol-induced conditioned place preference is expressed through a ventral tegmental area dependent mechanism. *Behav. Neurosci.* *119*: 213–23.

Becker, J.B. (1999). Gender differences in dopaminergic function in striatum and nucleus accumbens. *Pharmacol. Biochem. Behav.* *64*: 803–12.

Beckstead, M.J., Grandy, D.K., Wickman, K., and Williams, J.T. (2004). Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons. *Neuron* *42*: 939–46.

Berecki, G., McArthur, J.R., Cuny, H., Clark, R.J., and Adams, D.J. (2014). Differential Cav2.1 and Cav2.3 channel inhibition by baclofen and -conotoxin Vc1.1 via GABAB receptor activation. *J. Gen. Physiol.* *143*: 465–479.

Berman, D.M., Wilkie, T.M., and Gilman, A.G. (1996). GAIP and RGS4 are GTPase-activating proteins for the G(i) subfamily of G protein  $\alpha$  subunits. *Cell* *86*: 445–452.

- Bernard, V., and Bolam, J.P. (1998). Subcellular and subsynaptic distribution of the NR1 subunit of the NMDA receptor in the neostriatum and globus pallidus of the rat: co-localization at synapses with the GluR2/3 subunit of the AMPA receptor. *Eur. J. Neurosci.* *10*: 3721–36.
- Berner, L.A., Bocarsly, M.E., Hoebel, B.G., and Avena, N.M. (2009). Baclofen suppresses binge eating of pure fat but not a sugar-rich or sweet-fat diet. *Behav. Pharmacol.* *20*: 631–4.
- Berridge, C.W., Stratford, T.L., Foote, S.L., and Kelley, A.E. (1997). Distribution of dopamine beta-hydroxylase-like immunoreactive fibers within the shell subregion of the nucleus accumbens. *Synapse* *27*: 230–41.
- Berthoud, H.R. (2004). Mind versus metabolism in the control of food intake and energy balance. *Physiol. Behav.* *81*: 781–93.
- Berthoud, H.R. (2012). The neurobiology of food intake in an obesogenic environment. *Proc. Nutr. Soc.* *71*: 478–487.
- Bettler, B., Kaupmann, K., Mosbacher, J., and Gassmann, M. (2004). Molecular structure and physiological functions of GABA(B) receptors. *Physiol. Rev.* *84*: 835–67.
- Bettler, B., and Tiao, J.Y. (2006). Molecular diversity, trafficking and subcellular localization of GABAB receptors. *Pharmacol. Ther.* *110*: 533–43.
- Biermann, B., Ivankova-Susankova, K., Bradaia, A., Abdel Aziz, S., Besseyrias, V., Kapfhammer, J.P., et al. (2010). The Sushi domains of GABAB receptors function as axonal targeting signals. *J. Neurosci.* *30*: 1385–1394.
- Binet, V., Brajon, C., Le Corre, L., Acher, F., Pin, J.P., and Prézeau, L. (2004). The heptahelical domain of GABA(B2) is activated directly by CGP7930, a positive allosteric modulator of the GABA(B) receptor. *J. Biol. Chem.* *279*: 29085–91.
- Bischoff, S., Leonhard, S., Reymann, N., Schuler, V., Shigemoto, R., Kaupmann, K., et al. (1999). Spatial distribution of GABA(B)R1 receptor mRNA and binding sites in the rat brain. *J. Comp. Neurol.* *412*: 1–16.
- Blein, S., Ginham, R., Uhrin, D., Smith, B.O., Soares, D.C., Veltel, S., et al. (2004). Structural analysis of the complement control protein (CCP) modules of GABAB receptor 1a: Only one of the two CCP modules is compactly folded. *J. Biol. Chem.* *279*: 48292–48306.
- Blumer, K.J., and Thorner, J. (1990). Beta and gamma subunits of a yeast guanine nucleotide-binding protein are not essential for membrane association of the alpha subunit but are required for receptor coupling. *Proc. Natl. Acad. Sci. U. S. A.* *87*: 4363–4367.

Bolan, E.A., Kivell, B., Jaligam, V., O, M., Jayanthi, L.D., Han, Y., et al. (2007). D2 receptors regulate dopamine transporter function via an extracellular signal-regulated kinases 1 and 2-dependent and phosphoinositide 3 kinase-independent mechanism. *Mol. Pharmacol.* 71: 1222–32.

Bonanno, G., Fassio, A., Schmid, G., Severi, P., Sala, R., and Raiteri, M. (1997). Pharmacologically distinct GABAB receptors that mediate inhibition of GABA and glutamate release in human neocortex. *Br. J. Pharmacol.* 120: 60–4.

Bonanno, G., and Raiteri, M. (1993). gamma-Aminobutyric acid (GABA) autoreceptors in rat cerebral cortex and spinal cord represent pharmacologically distinct subtypes of the GABAB receptor. *J. Pharmacol. Exp. Ther.* 265: 765–770.

Bossert, J.M., Poles, G.C., Wihbey, K.A., Koya, E., and Shaham, Y. (2007). Differential effects of blockade of dopamine D1-family receptors in nucleus accumbens core or shell on reinstatement of heroin seeking induced by contextual and discrete cues. *J. Neurosci.* 27: 12655–12663.

Bowery, N.G., Bettler, B., Froestl, W., Gallagher, J.P., Marshall, F., Raiteri, M., et al. (2002). International Union of Pharmacology. XXXIII. Mammalian gamma-aminobutyric acid(B) receptors: structure and function. *Pharmacol. Rev.* 54: 247–64.

Bowery, N.G., Doble, A., Hill, D.R., Hudson, A.L., Shaw, J.S., and Turnbull, M.J. (1979). Baclofen: a selective agonist for a novel type of GABA receptor proceedings. *Br. J. Pharmacol.* 67: 444P–445P.

Bowery, N.G., and Hudson, A.L. (1979). gamma-Aminobutyric acid reduces the evoked release of [3H]-noradrenaline from sympathetic nerve terminals [proceedings]. *Br. J. Pharmacol.* 66: 108P.

Bowery, N.G., Hudson, A.L., and Price, G.W. (1987). GABAA and GABAB receptor site distribution in the rat central nervous system. *Neuroscience* 20: 365–83.

Bowery, N.G., and Smart, T.G. (2006). GABA and glycine as neurotransmitters: a brief history. *Br. J. Pharmacol.* 147 *Suppl* : S109–19.

Boyer, S.B., Clancy, S.M., Terunuma, M., Revilla-Sanchez, R., Thomas, S.M., Moss, S.J., et al. (2009). Direct interaction of GABAB receptors with M2 muscarinic receptors enhances muscarinic signaling. *J. Neurosci.* 29: 15796–15809.

Bräuner-Osborne, H., and Krogsgaard-Larsen, P. (1999). Functional pharmacology of cloned heterodimeric GABAB receptors expressed in mammalian cells. *Br. J. Pharmacol.* 128: 1370–1374.

- Brebner, K., Ahn, S., and Phillips, A.G. (2005a). Attenuation of d-amphetamine self-administration by baclofen in the rat: Behavioral and neurochemical correlates. *Psychopharmacology (Berl)*. 177: 409–417.
- Brebner, K., Froestl, W., Andrews, M., Phelan, R., and Roberts, D.C. (1999). The GABA(B) agonist CGP 44532 decreases cocaine self-administration in rats: demonstration using a progressive ratio and a discrete trials procedure. *Neuropharmacology* 38: 1797–804.
- Brebner, K., Phelan, R., and Roberts, D.C. (2000a). Effect of baclofen on cocaine self-administration in rats reinforced under fixed-ratio 1 and progressive-ratio schedules. *Psychopharmacology (Berl)*. 148: 314–21.
- Brebner, K., Phelan, R., and Roberts, D.C. (2000b). Intra-VTA baclofen attenuates cocaine self-administration on a progressive ratio schedule of reinforcement. *Pharmacol. Biochem. Behav.* 66: 857–62.
- Brebner, K., Wong, T.P., Liu, L., Liu, Y., Campsall, P., Gray, S., et al. (2005b). Nucleus accumbens long-term depression and the expression of behavioral sensitization. *Science* 310: 1340–3.
- Britt, J.P., and McGehee, D.S. (2008). Presynaptic opioid and nicotinic receptor modulation of dopamine overflow in the nucleus accumbens. *J. Neurosci.* 28: 1672–81.
- Broft, A.I., Spanos, A., Corwin, R.L., Mayer, L., Steinglass, J., Devlin, M.J., et al. (2007). Baclofen for binge eating: an open-label trial. *Int. J. Eat. Disord.* 40: 687–91.
- Brogden, R.N., Speight, T.M., and Avery, G.S. (1974). Baclofen: a preliminary report of its pharmacological properties and therapeutic efficacy in spasticity.
- Brown, D.A., and Higgins, A.J. (1979). Presynaptic effects of gamma-aminobutyric acid in isolated rat superior cervical ganglia [proceedings]. *Br. J. Pharmacol.* 66: 108P–109P.
- Buda-Levin, A., Wojnicki, F.H.E., and Corwin, R.L. (2005). Baclofen reduces fat intake under binge-type conditions. *Physiol. Behav.* 86: 176–184.
- Buonassisi, V., Sato, G., and Cohen, A.I. (1962). Hormone-producing cultures of adrenal and pituitary tumor origin. *Proc. Natl. Acad. Sci. U. S. A.* 48: 1184–90.
- Bussi eres, N., and Manira, A. El (1999). GABA(B) receptor activation inhibits N- and P/Q-type calcium channels in cultured lamprey sensory neurons. *Brain Res.* 847: 175–185.
- Cadoni, C., Solinas, M., and Chiara, G. Di (2000). Psychostimulant sensitization: differential changes in accumbal shell and core dopamine. *Eur. J. Pharmacol.* 388: 69–76.

- Callaghan, B., Haythornthwaite, A., Berecki, G., Clark, R.J., Craik, D.J., and Adams, D.J. (2008). Analgesic alpha-conotoxins Vc1.1 and Rg1A inhibit N-type calcium channels in rat sensory neurons via GABAB receptor activation. *J. Neurosci.* 28: 10943–51.
- Campbell, U.C., Lac, S.T., and Carroll, M.E. (1999). Effects of baclofen on maintenance and reinstatement of intravenous cocaine self-administration in rats. *Psychopharmacology (Berl)*. 143: 209–14.
- Cardozo, D.L., and Bean, B.P. (1995). Voltage-dependent calcium channels in rat midbrain dopamine neurons: modulation by dopamine and GABAB receptors. *J. Neurophysiol.* 74: 1137–48.
- Carlezon, W.A., and Chartoff, E.H. (2007). Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. *Nat. Protoc.* 2: 2987–2995.
- Cass, W.A., and Gerhardt, G.A. (1994). Direct in vivo evidence that D2 dopamine receptors can modulate dopamine uptake. *Neurosci. Lett.* 176: 259–63.
- Chalifoux, J.R., and Carter, A.G. (2010). GABAB Receptors Modulate NMDA Receptor Calcium Signals in Dendritic Spines. *Neuron* 66: 101–113.
- Chang, W., Tu, C., Cheng, Z., Rodriguez, L., Chen, T.H., Gassmann, M., et al. (2007). Complex formation with the Type B gamma-aminobutyric acid receptor affects the expression and signal transduction of the extracellular calcium-sensing receptor. Studies with HEK-293 cells and neurons. *J. Biol. Chem.* 282: 25030–25040.
- Charara, A, Heilman, T.C., Levey, A.L., and Smith, Y. (2000). Pre- and postsynaptic localization of GABA(B) receptors in the basal ganglia in monkeys. *Neuroscience* 95: 127–40.
- Chaudhri, N., Sahuque, L.L., Schairer, W.W., and Janak, P.H. (2010). Separable roles of the nucleus accumbens core and shell in context- and cue-induced alcohol-seeking. *Neuropsychopharmacology* 35: 783–791.
- Chen, B.T., Moran, K. A, Avshalumov, M. V., and Rice, M.E. (2006). Limited regulation of somatodendritic dopamine release by voltage-sensitive Ca channels contrasted with strong regulation of axonal dopamine release. *J. Neurochem.* 96: 645–55.
- Chen, B.T., Patel, J.C., Moran, K.A., and Rice, M.E. (2011). Differential calcium dependence of axonal versus somatodendritic dopamine release, with characteristics of both in the ventral tegmental area. *Front. Syst. Neurosci.* 5: 39.
- Chen, G., and van den Pol, A.N. (1998). Presynaptic GABAB autoreceptor modulation of P/Q-type calcium channels and GABA release in rat suprachiasmatic nucleus neurons. *J. Neurosci.* 18: 1913–1922.

- Chen, Y., Phillips, K., Minton, G., and Sher, E. (2005). GABA(B) receptor modulators potentiate baclofen-induced depression of dopamine neuron activity in the rat ventral tegmental area. *Br. J. Pharmacol.* *144*: 926–32.
- Cheng, Z., Tu, C., Rodriguez, L., Chen, T.H., Dvorak, M.M., Margeta, M., et al. (2007). Type B gamma-aminobutyric acid receptors modulate the function of the extracellular Ca<sup>2+</sup>-sensing receptor and cell differentiation in murine growth plate chondrocytes. *Endocrinology* *148*: 4984–4992.
- Chergui, K., Charléty, P.J., Akaoka, H., Saunier, C.F., Brunet, J.L., Buda, M., et al. (1993). Tonic activation of NMDA receptors causes spontaneous burst discharge of rat midbrain dopamine neurons in vivo. *Eur. J. Neurosci.* *5*: 137–44.
- Chergui, K., Suaud-Chagny, M.F., and Gonon, F. (1994). Nonlinear relationship between impulse flow, dopamine release and dopamine elimination in the rat brain in vivo. *Neuroscience* *62*: 641–5.
- Ciccarelli, A., Calza, A., Panzanelli, P., Concas, A., Giustetto, M., and Sassoè-Pognetto, M. (2012). Organization of GABAergic synaptic circuits in the rat ventral tegmental area. *PLoS One* *7*: e46250.
- Ciruela, F., Fernández-Dueñas, V., Sahlholm, K., Fernández-Alacid, L., Nicolau, J.C., Watanabe, M., et al. (2010). Evidence for oligomerization between GABAB receptors and GIRK channels containing the GIRK1 and GIRK3 subunits. *Eur. J. Neurosci.* *32*: 1265–1277.
- Clark, J.A., Mezey, E., Lam, A.S., and Bonner, T.I. (2000). Distribution of the GABA(B) receptor subunit gb2 in rat CNS. *Brain Res.* *860*: 41–52.
- Colantuoni, C., Schwenker, J., McCarthy, J., Rada, P., Ladenheim, B., Cadet, J.L., et al. (2001). Excessive sugar intake alters binding to dopamine and mu-opioid receptors in the brain. *Neuroreport* *12*: 3549–52.
- Colmers, W.F., and Williams, J.T. (1988). Pertussis toxin pretreatment discriminates between pre- and postsynaptic actions of baclofen in rat dorsal raphe nucleus in vitro. *Neurosci. Lett.* *93*: 300–306.
- Colombo, G., Agabio, R., Carai, M.A., Lobina, C., Pani, M., Reali, R., et al. (2000). Ability of baclofen in reducing alcohol intake and withdrawal severity: I--Preclinical evidence. *Alcohol. Clin. Exp. Res.* *24*: 58–66.
- Colombo, G., Serra, S., Brunetti, G., Atzori, G., Pani, M., Vacca, G., et al. The GABA(B) receptor agonists baclofen and CGP 44532 prevent acquisition of alcohol drinking behaviour in alcohol-preferring rats. *Alcohol* *37*: 499–503.

Conason, A.H., Brunstein Klomek, A., and Sher, L. (2006). Recognizing alcohol and drug abuse in patients with eating disorders. *QJM* 99: 335–9.

Cone, J.J., Chartoff, E.H., Potter, D.N., Ebner, S.R., and Roitman, M.F. (2013). Prolonged High Fat Diet Reduces Dopamine Reuptake without Altering DAT Gene Expression. *PLoS One* 8: e58251.

Congar, P., Bergevin, A., and Trudeau, L.E. (2002). D2 receptors inhibit the secretory process downstream from calcium influx in dopaminergic neurons: implication of K<sup>+</sup> channels. *J. Neurophysiol.* 87: 1046–56.

Cooke, J.E. (2010). Actions of isovaline and endogenous amino acids on inhibitory receptors in ventrobasal thalamus. The University of British Columbia.

Cooke, J.E., Mathers, D.A., and Puil, E. (2009). Isovaline causes inhibition by increasing potassium conductance in thalamic neurons. *Neuroscience* 164: 1235–43.

Cooke, J.E., Mathers, D.A., and Puil, E. (2012). R-Isovaline: a subtype-specific agonist at GABA(B)-receptors? *Neuroscience* 201: 85–95.

Corrigall, W.A., Coen, K.M., Adamson, K.L., Chow, B.L., and Zhang, J. (2000). Response of nicotine self-administration in the rat to manipulations of mu-opioid and gamma-aminobutyric acid receptors in the ventral tegmental area. *Psychopharmacology (Berl.)* 149: 107–14.

Corwin, R.L., Boan, J., Peters, K.F., and Ulbrecht, J.S. (2012). Baclofen reduces binge eating in a double-blind, placebo-controlled, crossover study. *Behav. Pharmacol.* 23: 616–25.

Cottone, P., Sabino, V., Steardo, L., and Zorrilla, E.P. (2008). Opioid-dependent anticipatory negative contrast and binge-like eating in rats with limited access to highly preferred food. *Neuropsychopharmacology* 33: 524–35.

Couey, J.J., Meredith, R.M., Spijker, S., Poorthuis, R.B., Smit, A.B., Brussaard, A.B., et al. (2007). Distributed network actions by nicotine increase the threshold for spike-timing-dependent plasticity in prefrontal cortex. *Neuron* 54: 73–87.

Couve, A, Thomas, P., Calver, a R., Hirst, W.D., Pangalos, M.N., Walsh, F.S., et al. (2002). Cyclic AMP-dependent protein kinase phosphorylation facilitates GABA(B) receptor-effector coupling. *Nat. Neurosci.* 5: 415–24.

Couve, A., Filippov, A.K., Connolly, C.N., Bettler, B., Brown, D.A., and Moss, S.J. (1998). Intracellular retention of recombinant GABA(B) receptors. *J. Biol. Chem.* 273: 26361–26367.

Couve, A., Kittler, J.T., Uren, J.M., Calver, A.R., Pangalos, M.N., Walsh, F.S., et al. (2001). Association of GABA(B) receptors and members of the 14-3-3 family of signaling proteins. *Mol. Cell. Neurosci.* 17: 317–28.

- Cragg, S.J. (2003). Variable dopamine release probability and short-term plasticity between functional domains of the primate striatum. *J. Neurosci.* 23: 4378–85.
- Cruz, H.G., Ivanova, T., Lunn, M.L., Stoffel, M., Slesinger, P.A., and Lüscher, C. (2004). Bi-directional effects of GABA(B) receptor agonists on the mesolimbic dopamine system. *Nat. Neurosci.* 7: 153–9.
- Cryan, J.F., Kelly, P.H., Chaperon, F., Gentsch, C., Mombereau, C., Lingenhoehl, K., et al. (2004). Behavioral characterization of the novel GABAB receptor-positive modulator GS39783 (N,N'-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine): anxiolytic-like activity without side effects associated with baclofen or benzodiazepines. *J. Pharmacol. Exp. Ther.* 310: 952–63.
- Cunningham, M.D., and Enna, S.J. (1996). Evidence for pharmacologically distinct GABAB receptors associated with cAMP production in rat brain. *Brain Res.* 720: 220–224.
- Cutting, D.A., and Jordan, C.C. (1975). Alternative approaches to analgesia: baclofen as a model compound. *Br. J. Pharmacol.* 54: 171–9.
- Dal Toso, R., Sommer, B., Ewert, M., Herb, A., Pritchett, D.B., Bach, A., et al. (1989). The dopamine D2 receptor: two molecular forms generated by alternative splicing. *EMBO J.* 8: 4025–34.
- Daoust, M., Saligaut, C., Lhuintre, J.P., Moore, N., Flipo, J.L., and Boismare, F. GABA transmission, but not benzodiazepine receptor stimulation, modulates ethanol intake by rats. *Alcohol* 4: 469–72.
- Dario, A., and Tomei, G. (2004). A benefit-risk assessment of baclofen in severe spinal spasticity. *Drug Saf.* 27: 799–818.
- David, M., Richer, M., Mamarbachi, A.M., Villeneuve, L.R., Dupré, D.J., and Hebert, T.E. (2006). Interactions between GABA-B1 receptors and Kir 3 inwardly rectifying potassium channels. *Cell. Signal.* 18: 2172–2181.
- Davis, J.F., Tracy, A.L., Schurdak, J.D., Tschöp, M.H., Lipton, J.W., Clegg, D.J., et al. (2008). Exposure to elevated levels of dietary fat attenuates psychostimulant reward and mesolimbic dopamine turnover in the rat. *Behav. Neurosci.* 122: 1257–1263.
- Dawson, V.L., Dawson, T.M., Filloux, F.M., and Wamsley, J.K. (1988). Evidence for dopamine D-2 receptors on cholinergic interneurons in the rat caudate-putamen. *Life Sci.* 42: 1933–9.
- Day, J.J., Roitman, M.F., Wightman, R.M., and Carelli, R.M. (2007). Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nat. Neurosci.* 10: 1020–8.

- DeBoer, P., and Westerink, B.H. (1994). GABAergic modulation of striatal cholinergic interneurons: an in vivo microdialysis study. *J. Neurochem.* 62: 70–5.
- Deisz, R.A., Billard, J.M., and Zieglgänsberger, W. (1993). Pre- and postsynaptic GABAB receptors of rat neocortical neurons differ in their pharmacological properties. *Neurosci. Lett.* 154: 209–212.
- Delmas, P., Brown, D.A., Dayrell, M., Abogadie, F.C., Caulfield, M.P., and Buckley, N.J. (1998). On the role of endogenous G-protein beta gamma subunits in N-type Ca<sup>2+</sup> current inhibition by neurotransmitters in rat sympathetic neurones. *J. Physiol.* 506: 319–329.
- Deng, P.Y., Xiao, Z., Yang, C., Rojanathammanee, L., Grisanti, L., Watt, J., et al. (2009). GABAB Receptor Activation Inhibits Neuronal Excitability and Spatial Learning in the Entorhinal Cortex by Activating TREK-2 K<sup>+</sup> Channels. *Neuron* 63: 230–243.
- Deterre, P., Bigay, J., Pfister, C., and Chabre, M. (1984). Guanine nucleotides and magnesium dependence of the association states of the subunits of transducin. *FEBS Lett.* 178: 228–232.
- Di Chiara, G., and Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. U. S. A.* 85: 5274–8.
- Dickinson, S.D., Sabeti, J., Larson, G. A., Giardina, K., Rubinstein, M., Kelly, M.A., et al. (1999). Dopamine D2 receptor-deficient mice exhibit decreased dopamine transporter function but no changes in dopamine release in dorsal striatum. *J. Neurochem.* 72: 148–56.
- Ding, J.B., Guzman, J.N., Peterson, J.D., Goldberg, J.A., and Surmeier, D.J. (2010). Thalamic gating of corticostriatal signaling by cholinergic interneurons. *Neuron* 67: 294–307.
- Diversé-Pierluissi, M., Remmers, A.E., Neubig, R.R., and Dunlap, K. (1997). Novel form of crosstalk between G protein and tyrosine kinase pathways. *Proc. Natl. Acad. Sci. U. S. A.* 94: 5417–5421.
- Dobrovitsky, V., Pimentel, P., Duarte, A., Froestl, W., Stellar, J.R., and Trzcińska, M. (2002). CGP 44532, a GABAB receptor agonist, is hedonically neutral and reduces cocaine-induced enhancement of reward. *Neuropharmacology* 42: 626–32.
- Doupnik, C.A. (2008). GPCR-Kir channel signaling complexes: defining rules of engagement. *J. Recept. Signal Transduct. Res.* 28: 83–91.
- Doupnik, C.A., Davidson, N., Lester, H.A., and Kofuji, P. (1997). RGS proteins reconstitute the rapid gating kinetics of gbetagamma-activated inwardly rectifying K<sup>+</sup> channels. *Proc. Natl. Acad. Sci. U. S. A.* 94: 10461–10466.

Dousmanis, A.G., and Pennefather, P.S. (1992). Inwardly rectifying potassium conductances in AtT-20 clonal pituitary cells. *Pflugers Arch.* 422: 98–104.

Dunlap, K., and Fischbach, G.D. (1981). Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurones. *J. Physiol.* 317: 519–535.

Dupuis, D.S., Relkovic, D., Lhuillier, L., Mosbacher, J., and Kaupmann, K. (2006). Point mutations in the transmembrane region of GABAB2 facilitate activation by the positive modulator N,N'-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) in the absence of the GABAB1 subunit. *Mol. Pharmacol.* 70: 2027–2036.

Dutar, P., and Nicoll, R.A. (1988). Pre- and postsynaptic GABAB receptors in the hippocampus have different pharmacological properties. *Neuron* 1: 585–591.

Duthey, B., Caudron, S., Perroy, J., Bettler, B., Fagni, L., Pin, J.P., et al. (2002). A single subunit (GB2) is required for G-protein activation by the heterodimeric GABAB receptor. *J. Biol. Chem.* 277: 3236–3241.

Ebenezer, I.S. (1990). The effect of intracerebroventricular administration of baclofen on food intake in rats. *Neuroreport* 1: 73–76.

Ebenezer, I.S., and Pringle, A.K. (1992). The effect of systemic administration of baclofen on food intake in rats. *Neuropharmacology* 31: 39–42.

el Ganouni, S., Forni, C., and Nieoullon, A. (1987). In vitro and in vivo characterization of the properties of a multifiber carbon electrode allowing long-term electrochemical detection of dopamine in freely moving animals. *Brain Res.* 404: 239–56.

Enna, S.J., and McCarron, K.E. (2006). The role of GABA in the mediation and perception of pain. *Adv. Pharmacol.* 54: 1–27.

Erhardt, S., Mathé, J.M., Chergui, K., Engberg, G., and Svensson, T.H. (2002). GABA(B) receptor-mediated modulation of the firing pattern of ventral tegmental area dopamine neurons in vivo. *Naunyn. Schmiedeberg's Arch. Pharmacol.* 365: 173–80.

Fadda, P., Scherma, M., Fresu, A., Collu, M., and Fratta, W. (2003). Baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the nucleus accumbens of rat. *Synapse* 50: 1–6.

Fairfax, B.P., Pitcher, J.A., Scott, M.G.H., Calver, A.R., Pangalos, M.N., Moss, S.J., et al. (2004). Phosphorylation and Chronic Agonist Treatment Atypically Modulate GABA B Receptor Cell Surface Stability. *J. Biol. Chem.* 279: 12565–12573.

Fajardo-Serrano, A., Wydeven, N., Young, D., Watanabe, M., Shigemoto, R., Martemyanov, K.A., et al. (2013). Association of Rgs7/G $\beta$ 5 complexes with Girk channels and GABAB receptors in hippocampal CA1 pyramidal neurons. *Hippocampus* 23: 1231–45.

Fallon, J.H. (1988). Topographic organization of ascending dopaminergic projections. *Ann. N. Y. Acad. Sci.* 537: 1–9.

Fassio, A., Bonanno, G., Cavazzani, P., and Raiteri, M. (1994). Characterization of the GABA autoreceptor in human neocortex as a pharmacological subtype of the GABAB receptor. *Eur. J. Pharmacol.* 263: 311–314.

Fattore, L., Cossu, G., Martellotta, M.C., and Fratta, W. (2002) Baclofen antagonizes intravenous self-administration of nicotine in mice and rats. *Alcohol* 37: 495–8.

Febo, M., and Segarra, A.C. (2004). Cocaine alters GABA(B)-mediated G-protein activation in the ventral tegmental area of female rats: modulation by estrogen. *Synapse* 54: 30–6.

Fenton, H.M., and Liebman, J.M. (1982). Self-stimulation response decrement patterns differentiate clonidine, baclofen and dopamine antagonists from drugs causing performance deficit. *Pharmacol. Biochem. Behav.* 17: 1207–12.

Fernández-Alacid, L., Aguado, C., Ciruela, F., Martín, R., Colón, J., Cabañero, M.J., et al. (2009). Subcellular compartment-specific molecular diversity of pre- and post-synaptic GABAB-activated GIRK channels in Purkinje cells. *J. Neurochem.* 110: 1363–1376.

Ferris, M.J., Calipari, E.S., Yorgason, J.T., and Jones, S.R. (2013). Examining the complex regulation and drug-induced plasticity of dopamine release and uptake using voltammetry in brain slices. *ACS Chem. Neurosci.* 4: 693–703.

Filip, M., Frankowska, M., and Przegaliński, E. (2007). Effects of GABAB receptor antagonist, agonists and allosteric positive modulator on the cocaine-induced self-administration and drug discrimination. *Eur. J. Pharmacol.* 574: 148–157.

Filip, M., Frankowska, M., Sadakierska-Chudy, A., Suder, A., Szumiec, Ł., Mierzejewski, P., et al. (2015). GABAB receptors as a therapeutic strategy in substance use disorders: Focus on positive allosteric modulators. *Neuropharmacology* 88: 36–47.

Filip, M., and Siwanowicz, J. Implication of the nucleus accumbens shell, but not core, in the acute and sensitizing effects of cocaine in rats. *Pol. J. Pharmacol.* 53: 459–66.

Floresco, S.B., McLaughlin, R.J., and Haluk, D.M. (2008). Opposing roles for the nucleus accumbens core and shell in cue-induced reinstatement of food-seeking behavior. *Neuroscience* 154: 877–884.

- Floresco, S.B., West, A.R., Ash, B., Moore, H., and Grace, A.A. (2003). Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nat. Neurosci.* 6: 968–73.
- Foll, B. Le, Wertheim, C.E., and Goldberg, S.R. (2008). Effects of baclofen on conditioned rewarding and discriminative stimulus effects of nicotine in rats. *Neurosci. Lett.* 443: 236–240.
- Ford, C.P., Beckstead, M.J., and Williams, J.T. (2007). Kappa opioid inhibition of somatodendritic dopamine inhibitory postsynaptic currents. *J. Neurophysiol.* 97: 883–91.
- Ford, C.P., Gantz, S.C., Phillips, P.E.M., and Williams, J.T. (2010). Control of extracellular dopamine at dendrite and axon terminals. *J. Neurosci.* 30: 6975–83.
- Fowler, C.E., Aryal, P., Suen, K.F., and Slesinger, P.A. (2007). Evidence for association of GABA(B) receptors with Kir3 channels and regulators of G protein signalling (RGS4) proteins. *J. Physiol.* 580: 51–65.
- Franek, M., Pagano, A., Kaupmann, K., Bettler, B., Pin, J.P., and Blahos, J. (1999). The heteromeric GABA-B receptor recognizes G-protein alpha subunit C-termini. *Neuropharmacology* 38: 1657–66.
- Frankowska, M., Filip, M., and Przegaliński, E. (2007). Effects of GABAB receptor ligands in animal tests of depression and anxiety. *Pharmacol. Reports* 59: 645–655.
- Frankowska, M., Nowak, E., and Filip, M. (2009). Effects of GABAB receptor agonists on cocaine hyperlocomotor and sensitizing effects in rats. *Pharmacol. Reports* 61: 1042–1049.
- Frankowska, M., Wydra, K., Faron-Górecka, A., Zaniewska, M., Kuśmider, M., Dziejicka-Wasylewska, M., et al. (2008). Neuroadaptive changes in the rat brain GABAB receptors after withdrawal from cocaine self-administration. *Eur. J. Pharmacol.* 599: 58–64.
- Froestl, W. (2010). Chemistry and pharmacology of GABAB receptor ligands. *Adv. Pharmacol.* 58: 19–62.
- Froestl, W., Mickel, S.J., Hall, R.G., Sprecher, G. von, Strub, D., Baumann, P.A., et al. (1995). Phosphinic acid analogues of GABA. 1. New potent and selective GABAB agonists. *J. Med. Chem.* 38: 3297–3312.
- Fu, Z., Yang, H., Xiao, Y., Zhao, G., and Huang, H. (2012). The  $\gamma$ -aminobutyric acid type B (GABAB) receptor agonist baclofen inhibits morphine sensitization by decreasing the dopamine level in rat nucleus accumbens. *Behav. Brain Funct.* 8: 20.

- Fulton, S., Thibault, D., Mendez, J.A., Lahaie, N., Tirotta, E., Borrelli, E., et al. (2011). Contribution of Kv1.2 voltage-gated potassium channel to D2 autoreceptor regulation of axonal dopamine overflow. *J. Biol. Chem.* *286*: 9360–72.
- Fumagalli, F., Jones, S., Bossé, R., Jaber, M., Giros, B., Missale, C., et al. (1998). Inactivation of the dopamine transporter reveals essential roles of dopamine in the control of locomotion, psychostimulant response, and pituitary function. *Adv. Pharmacol.* *42*: 179–82.
- Furth, J., Gadsen, E.L., and Upton, A.C. (1953). ACTH secreting transplantable pituitary tumors. *Proc. Soc. Exp. Biol. Med.* *84*: 253–4.
- Gähwiler, B.H., and Brown, D.A. (1985). GABAB-receptor-activated K<sup>+</sup> current in voltage-clamped CA3 pyramidal cells in hippocampal cultures. *Proc. Natl. Acad. Sci. U. S. A.* *82*: 1558–1562.
- Galvez, T., Duthey, B., Kniazeff, J., Blahos, J., Rovelli, G., Bettler, B., et al. (2001). Allosteric interactions between GB1 and GB2 subunits are required for optimal GABAB receptor function. *EMBO J.* *20*: 2152–2159.
- Galvez, T., Parmentier, M.L., Joly, C., Malitschek, B., Kaupmann, K., Kuhn, R., et al. (1999). Mutagenesis and modeling of the GABAB receptor extracellular domain support a venus flytrap mechanism for ligand binding. *J. Biol. Chem.* *274*: 13362–9.
- Galvez, T., Prezeau, L., Milioti, G., Franek, M., Joly, C., Froestl, W., et al. (2000a). Mapping the agonist-binding site of GABAB type 1 subunit sheds light on the activation process of GABAB receptors. *J. Biol. Chem.* *275*: 41166–74.
- Galvez, T., Urwyler, S., Prézeau, L., Mosbacher, J., Joly, C., Malitschek, B., et al. (2000b). Ca<sup>2+</sup> requirement for high-affinity gamma-aminobutyric acid (GABA) binding at GABA(B) receptors: involvement of serine 269 of the GABA(B)R1 subunit. *Mol. Pharmacol.* *57*: 419–426.
- Gan, J.O., Walton, M.E., and Phillips, P.E.M. (2010). Dissociable cost and benefit encoding of future rewards by mesolimbic dopamine. *Nat. Neurosci.* *13*: 25–7.
- Garabedian-Ruffalo, S.M., and Ruffalo, R.L. (1985). Adverse effects secondary to baclofen withdrawal. *Drug Intell. Clin. Pharm.* *19*: 304–6.
- Garris, P.A., Collins, L.B., Jones, S.R., and Wightman, R.M. (1993). Evoked extracellular dopamine in vivo in the medial prefrontal cortex. *J. Neurochem.* *61*: 637–47.
- Gassmann, M., Shaban, H., Vigot, R., Sansig, G., Haller, C., Barbieri, S., et al. (2004). Redistribution of GABAB(1) protein and atypical GABAB responses in GABAB(2)-deficient mice. *J. Neurosci.* *24*: 6086–6097.

- Geiger, B.M., Haburcak, M., Avena, N.M., Moyer, M.C., Hoebel, B.G., and Pothos, E.N. (2009). Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. *Neuroscience* 159: 1193–9.
- Geng, Y., Bush, M., Mosyak, L., Wang, F., and Fan, Q.R. (2013). Structural mechanism of ligand activation in human GABA(B) receptor. *Nature* 504: 254–9.
- Geng, Y., Xiong, D., Mosyak, L., Malito, D.L., Kniazeff, J., Chen, Y., et al. (2012). Structure and functional interaction of the extracellular domain of human GABAB receptor GBR2. *Nat. Neurosci.* 15: 970–978.
- Gerfen, C.R., and Surmeier, D.J. (2011). Modulation of striatal projection systems by dopamine. *Annu. Rev. Neurosci.* 34: 441–66.
- Gosnell, B.A. (2005). Sucrose intake enhances behavioral sensitization produced by cocaine. *Brain Res.* 1031: 194–201.
- Grace, A.A., and Bunney, B.S. (1984a). The control of firing pattern in nigral dopamine neurons: burst firing. *J. Neurosci.* 4: 2877–90.
- Grace, A.A., and Bunney, B.S. (1984b). The control of firing pattern in nigral dopamine neurons: single spike firing. *J. Neurosci.* 4: 2866–76.
- Grampp, T., Notz, V., Broll, I., Fischer, N., and Benke, D. (2008). Constitutive, agonist-accelerated, recycling and lysosomal degradation of GABAB receptors in cortical neurons. *Mol. Cell. Neurosci.* 39: 628–637.
- Grampp, T., Sauter, K., Markovic, B., and Benke, D. (2007). gamma-Aminobutyric acid type B receptors are constitutively internalized via the clathrin-dependent pathway and targeted to lysosomes for degradation. *J. Biol. Chem.* 282: 24157–24165.
- Green, E., Jacobson, A., Haase, L., and Murphy, C. (2011). Reduced nucleus accumbens and caudate nucleus activation to a pleasant taste is associated with obesity in older adults. *Brain Res.* 1386: 109–17.
- Gu, H., Wall, S.C., and Rudnick, G. (1994). Stable expression of biogenic amine transporters reveals differences in inhibitor sensitivity, kinetics, and ion dependence. *J. Biol. Chem.* 269: 7124–30.
- Guetg, N., Seddik, R., Vigot, R., Turecek, R., Gassmann, M., Vogt, K.E., et al. (2009). The GABAB1a isoform mediates heterosynaptic depression at hippocampal mossy fiber synapses. *J. Neurosci.* 29: 1414–1423.

Guyon, A., and Leresche, N. (1995). Modulation by different GABAB receptor types of voltage-activated calcium currents in rat thalamocortical neurones. *J. Physiol.* 485 ( Pt 1: 29–42.

Hajnal, A., Margas, W.M., and Covasa, M. (2008). Altered dopamine D2 receptor function and binding in obese OLETF rat. *Brain Res. Bull.* 75: 70–6.

Halbout, B., Quarta, D., Valerio, E., Heidbreder, C. A., and Hutcheson, D.M. (2011). The GABA-B positive modulator GS39783 decreases psychostimulant conditioned-reinforcement and conditioned-reward. *Addict. Biol.* 16: 416–27.

Hamdi, A., Porter, J., and Prasad, C. (1992). Decreased striatal D2 dopamine receptors in obese Zucker rats: changes during aging. *Brain Res.* 589: 338–40.

Hannan, S., Wilkins, M.E., Dehghani-Tafti, E., Thomas, P., Baddeley, S.M., and Smart, T.G. (2011). Gamma-aminobutyric acid type B (GABA(B)) receptor internalization is regulated by the R2 subunit. *J. Biol. Chem.* 286: 24324–24335.

Hannan, S., Wilkins, M.E., and Smart, T.G. (2012). Sushi domains confer distinct trafficking profiles on GABAB receptors. *Proc. Natl. Acad. Sci. U. S. A.* 109: 12171–6.

Harayama, N., Shibuya, I., Tanaka, K., Kabashima, N., Ueta, Y., and Yamashita, H. (1998). Inhibition of N- and P/Q-type calcium channels by postsynaptic GABA(B) receptor activation in rat supraoptic neurones. *J. Physiol.* 509: 371–383.

Harrison, C., and Traynor, J.R. (2003). The [35S]GTPgammaS binding assay: approaches and applications in pharmacology. *Life Sci.* 74: 489–508.

Havlickova, M., Prezeau, L., Duthey, B., Bettler, B., Pin, J.P., and Blahos, J. (2002). The intracellular loops of the GB2 subunit are crucial for G-protein coupling of the heteromeric gamma-aminobutyrate B receptor. *Mol. Pharmacol.* 62: 343–350.

Hawrot, E., Xiao, Y., Shi, Q.L., Norman, D., Kirkitadze, M., and Barlow, P.N. (1998). Demonstration of a tandem pair of complement protein modules in GABA(B) receptor 1a. *FEBS Lett.* 432: 103–108.

Hearing, M., Kotecki, L., Marron Fernandez de Velasco, E., Fajardo-Serrano, A., Chung, H.J., Luján, R., et al. (2013). Repeated Cocaine Weakens GABAB-Girk Signaling in Layer 5/6 Pyramidal Neurons in the Prelimbic Cortex. *Neuron* 80: 159–70.

Hedou, G., Feldon, J., and Heidbreder, C.A. (1999). Effects of cocaine on dopamine in subregions of the rat prefrontal cortex and their efferents to subterritories of the nucleus accumbens. *Eur. J. Pharmacol.* 372: 143–55.

- Heidbreder, C.A., Hedou, G., and Feldon, J. (1999). Behavioral neurochemistry reveals a new functional dichotomy in the shell subregion of the nucleus accumbens. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 23: 99–132.
- Heien, M.L.A. V, Phillips, P.E.M., Stuber, G.D., Seipel, A.T., and Wightman, R.M. (2003). Overoxidation of carbon-fiber microelectrodes enhances dopamine adsorption and increases sensitivity. *Analyst* 128: 1413–9.
- Herlitze, S., Garcia, D.E., Mackie, K., Hille, B., Scheuer, T., and Catterall, W.A. (1996). Modulation of Ca<sup>2+</sup> channels by G-protein beta gamma subunits. *Nature* 380: 258–262.
- Higgs, S., and Barber, D.J. (2004). Effects of baclofen on feeding behaviour examined in the runway. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 28: 405–8.
- Hill, D.R. (1985). GABAB receptor modulation of adenylate cyclase activity in rat brain slices. *Br. J. Pharmacol.* 84: 249–257.
- Hill, D.R., and Bowery, N.G. (1981). 3H-baclofen and 3H-GABA bind to bicuculline-insensitive GABA B sites in rat brain. *Nature* 290: 149–52.
- Hodos, W. (1961). Progressive ratio as a measure of reward strength. *Science* 134: 943–944.
- Huang, X.F., Yu, Y., Zavitsanou, K., Han, M., and Storlien, L. (2005). Differential expression of dopamine D2 and D4 receptor and tyrosine hydroxylase mRNA in mice prone, or resistant, to chronic high-fat diet-induced obesity. *Brain Res. Mol. Brain Res.* 135: 150–61.
- Hunt, T.W., Fields, T.A., Casey, P.J., and Peralta, E.G. (1996). RGS10 is a selective activator of G<sub>αi</sub> GTPase activity. *Nature* 383: 175–177.
- Huston, E., Cullen, G.P., Burley, J.R., and Dolphin, A.C. (1995). The involvement of multiple calcium channel sub-types in glutamate release from cerebellar granule cells and its modulation by GABAB receptor activation. *Neuroscience* 68: 465–478.
- Huynh, T.G., Cuny, H., Slesinger, P.A., and Adams, D.J. (2015). Novel mechanism of voltage-gated N-type (Cav2.2) calcium channel inhibition revealed through α-conotoxin Vc1.1 activation of the GABA(B) receptor. *Mol. Pharmacol.* 87: 240–50.
- Hyland, B.I., Reynolds, J.N.J., Hay, J., Perk, C.G., and Miller, R. (2002). Firing modes of midbrain dopamine cells in the freely moving rat. *Neuroscience* 114: 475–92.
- Imperato, A., and Di Chiara, G. (1988). Effects of locally applied D-1 and D-2 receptor agonists and antagonists studied with brain dialysis. *Eur. J. Pharmacol.* 156: 385–93.

Isaacson, J.S., Solis, J.M., and Nicoll, R.A. (1993). Local and diffuse synaptic actions of GABA in the hippocampus. *Neuron* 10: 165–175.

Isomoto, S., Kaibara, M., Sakurai-Yamashita, Y., Nagayama, Y., Uezono, Y., Yano, K., et al. (1998). Cloning and tissue distribution of novel splice variants of the rat GABAB receptor. *Biochem. Biophys. Res. Commun.* 253: 10–15.

Ivankova, K., Turecek, R., Fritzius, T., Seddik, R., Prezeau, L., Comps-Agrar, L., et al. (2013). Up-regulation of GABAB receptor signaling by constitutive assembly with the K<sup>+</sup> channel tetramerization domain-containing protein 12 (KCTD12). *J. Biol. Chem.* 288: 24848–24856.

Jabourian, M., Venance, L., Bourgoïn, S., Ozon, S., Pérez, S., Godeheu, G., et al. (2005). Functional mu opioid receptors are expressed in cholinergic interneurons of the rat dorsal striatum: territorial specificity and diurnal variation. *Eur. J. Neurosci.* 21: 3301–3309.

Jayaram, P., and Steketee, J.D. (2004). Effects of repeated cocaine on medial prefrontal cortical GABAB receptor modulation of neurotransmission in the mesocorticolimbic dopamine system. *J. Neurochem.* 90: 839–47.

Jelacic, T.M., Kennedy, M.E., Wickman, K., and Clapham, D.E. (2000). Functional and biochemical evidence for G-protein-gated inwardly rectifying K<sup>+</sup> (GIRK) channels composed of GIRK2 and GIRK3. *J. Biol. Chem.* 275: 36211–36216.

Johnson, P.M., and Kenny, P.J. (2010). Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat. Neurosci.* 13: 635–41.

Johnson, S.W., and North, R.A. (1992). Two types of neurone in the rat ventral tegmental area and their synaptic inputs. *J. Physiol.* 450: 455–68.

Jones, K.A., Borowsky, B., Tamm, J.A., Craig, D.A., Durkin, M.M., Dai, M., et al. (1998a). GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. *Nature* 396: 674–9.

Jones, S.R., Gainetdinov, R.R., Wightman, R.M., and Caron, M.G. (1998b). Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. *J. Neurosci.* 18: 1979–86.

Kanaide, M., Uezono, Y., Matsumoto, M., Hojo, M., Ando, Y., Sudo, Y., et al. (2007). Desensitization of GABA(B) receptor signaling by formation of protein complexes of GABA(B)2 subunit with GRK4 or GRK5. *J. Cell. Physiol.* 210: 237–45.

Kaplan, G.B., Leite-Morris, K.A., Joshi, M., Shoeb, M.H., and Carey, R.J. (2003). Baclofen inhibits opiate-induced conditioned place preference and associated induction of Fos in cortical and limbic regions. *Brain Res.* 987: 122–5.

Kaupmann, K., Huggel, K., Heid, J., Flor, P.J., Bischoff, S., Mickel, S.J., et al. (1997). Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature* 386: 239–246.

Kaupmann, K., Malitschek, B., Schuler, V., Heid, J., Froestl, W., Beck, P., et al. (1998). GABA(B)-receptor subtypes assemble into functional heteromeric complexes. *Nature* 396: 683–7.

Klitenick, M. A., DeWitte, P., and Kalivas, P.W. (1992). Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: an in vivo microdialysis study. *J. Neurosci.* 12: 2623–32.

Kofuji, P., Davidson, N., and Lester, H.A. (1995). Evidence that neuronal G-protein-gated inwardly rectifying K<sup>+</sup> channels are activated by G beta gamma subunits and function as heteromultimers. *Proc. Natl. Acad. Sci. U. S. A.* 92: 6542–6546.

Komendantov, A.O., Komendantova, O.G., Johnson, S.W., and Canavier, C.C. (2004). A modeling study suggests complementary roles for GABAA and NMDA receptors and the SK channel in regulating the firing pattern in midbrain dopamine neurons. *J. Neurophysiol.* 91: 346–57.

Krnjević, K., and Schwartz, S. (1967). The action of gamma-aminobutyric acid on cortical neurones. *Exp. Brain Res.* 3: 320–336.

Kubo, Y., Reuveny, E., Slesinger, P.A., Jan, Y.N., and Jan, L.Y. (1993). Primary structure and functional expression of a rat G-protein-coupled muscarinic potassium channel. *Nature* 364: 802–806.

Kulik, A., Vida, I., Luján, R., Haas, C.A., López-Bendito, G., Shigemoto, R., et al. (2003). Subcellular localization of metabotropic GABA(B) receptor subunits GABA(B1a/b) and GABA(B2) in the rat hippocampus. *J. Neurosci.* 23: 11026–11035.

Kuner, R., Köhr, G., Grünewald, S., Eisenhardt, G., Bach, A., and Kornau, H.C. (1999). Role of heteromer formation in GABAB receptor function. *Science* 283: 74–7.

Kuramoto, N., Wilkins, M.E., Fairfax, B.P., Revilla-Sanchez, R., Terunuma, M., Tamaki, K., et al. (2007). Phospho-Dependent Functional Modulation of GABAB Receptors by the Metabolic Sensor AMP-Dependent Protein Kinase. *Neuron* 53: 233–247.

Kushner, S.A., and Unterwald, E.M. (2001). Chronic cocaine administration decreases the functional coupling of GABA(B) receptors in the rat ventral tegmental area as measured by baclofen-stimulated 35S-GTPgammaS binding. *Life Sci.* 69: 1093–102.

Kwok-Keung Fung, B., and Stryer, L. (1980). Photolyzed rhodopsin catalyzes the exchange of GTP for bound GDP in retinal rod outer segments. *Proc. Natl. Acad. Sci. U. S. A.* 77: 2500–2504.

Labouèbe, G., Lomazzi, M., Cruz, H.G., Creton, C., Luján, R., Li, M., et al. (2007). RGS2 modulates coupling between GABAB receptors and GIRK channels in dopamine neurons of the ventral tegmental area. *Nat. Neurosci.* *10*: 1559–68.

Lacey, C.J., Boyes, J., Gerlach, O., Chen, L., Magill, P.J., and Bolam, J.P. (2005). GABAB receptors at glutamatergic synapses in the rat striatum. *Neuroscience* *136*: 1083–1095.

Ladera, C., del Carmen Godino, M., José Cabañero, M., Torres, M., Watanabe, M., Luján, R., et al. (2008). Pre-synaptic GABA receptors inhibit glutamate release through GIRK channels in rat cerebral cortex. *J. Neurochem.* *107*: 1506–1517.

La Fleur, S.E., Luijendijk, M.C.M., van der Zwaal, E.M., Brans, M.A.D., and Adan, R.A.H. (2014). The snacking rat as model of human obesity: effects of a free-choice high-fat high-sugar diet on meal patterns. *Int. J. Obes. (Lond).* *38*: 643–9.

Laviv, T., Vertkin, I., Berdichevsky, Y., Fogel, H., Riven, I., Bettler, B., et al. (2011). Compartmentalization of the GABAB receptor signaling complex is required for presynaptic inhibition at hippocampal synapses. *J. Neurosci.* *31*: 12523–32.

Leaney, J.L. (2003). Contribution of Kir3.1, Kir3.2A and Kir3.2C subunits to native G protein-gated inwardly rectifying potassium currents in cultured hippocampal neurons. *Eur. J. Neurosci.* *18*: 2110–2118.

Lehmann, A., Antonsson, M., Holmberg, A.A., Blackshaw, L.A., Brändén, L., Bräuner-Osborne, H., et al. (2009). (R)-(3-amino-2-fluoropropyl) phosphinic acid (AZD3355), a novel GABAB receptor agonist, inhibits transient lower esophageal sphincter relaxation through a peripheral mode of action. *J. Pharmacol. Exp. Ther.* *331*: 504–512.

Lesage, F., Duprat, F., Fink, M., Guillemare, E., Coppola, T., Lazdunski, M., et al. (1994). Cloning provides evidence for a family of inward rectifier and G-protein coupled K<sup>+</sup> channels in the brain. *FEBS Lett.* *353*: 37–42.

Li, S.M., Yin, L.L., Ren, Y.H., Pan, L.S., and Zheng, J.W. (2001). GABA(B) receptor agonist baclofen attenuates the development and expression of d-methamphetamine-induced place preference in rats. *Life Sci.* *70*: 349–56.

Li, Y., and Stern, J.E. (2004). Activation of postsynaptic GABAB receptors modulate the firing activity of supraoptic oxytocin and vasopressin neurones: Role of calcium channels. *J. Neuroendocrinol.* *16*: 119–130.

Liang, J.H., Chen, F., Krstew, E., Cowen, M.S., Carroll, F.Y., Crawford, D., et al. (2006). The GABA(B) receptor allosteric modulator CGP7930, like baclofen, reduces operant self-administration of ethanol in alcohol-preferring rats. *Neuropharmacology* *50*: 632–9.

- Lindgren, N., Xu, Z.Q., Herrera-Marschitz, M., Haycock, J., Hökfelt, T., and Fisone, G. (2001). Dopamine D(2) receptors regulate tyrosine hydroxylase activity and phosphorylation at Ser40 in rat striatum. *Eur. J. Neurosci.* *13*: 773–80.
- Lingenhoehl, K., Brom, R., Heid, J., Beck, P., Froestl, W., Kaupmann, K., et al. (1999). Gamma-hydroxybutyrate is a weak agonist at recombinant GABA(B) receptors. *Neuropharmacology* *38*: 1667–73.
- Liu, J., Maurel, D., Etzol, S., Brabet, I., Ansanay, H., Pin, J.P., et al. (2004). Molecular Determinants Involved in the Allosteric Control of Agonist Affinity in the GABAB Receptor by the GABAB2 Subunit. *J. Biol. Chem.* *279*: 15824–15830.
- Luján, R., Shigemoto, R., Kulik, A., and Juiz, J.M. (2004). Localization of the GABAB receptor 1a/b subunit relative to glutamatergic synapses in the dorsal cochlear nucleus of the rat. *J. Comp. Neurol.* *475*: 36–46.
- Lüscher, C., Jan, L.Y., Stoffel, M., Malenka, R.C., and Nicoll, R.A. (1997). G protein-coupled inwardly rectifying K<sup>+</sup> channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron* *19*: 687–695.
- Maccioni, P., Fantini, N., Froestl, W., Carai, M.A.M., Gessa, G.L., and Colombo, G. (2008). Specific reduction of alcohol's motivational properties by the positive allosteric modulator of the GABAB receptor, GS39783--comparison with the effect of the GABAB receptor direct agonist, baclofen. *Alcohol. Clin. Exp. Res.* *32*: 1558–64.
- Maccioni, P., Pes, D., Orrù, A., Froestl, W., Gessa, G.L., Carai, M.A.M., et al. (2007). Reducing effect of the positive allosteric modulator of the GABA(B) receptor, GS39,783, on alcohol self-administration in alcohol-preferring rats. *Psychopharmacology (Berl)*. *193*: 171–8.
- Maccioni, P., Serra, S., Vacca, G., Orrù, A., Pes, D., Agabio, R., et al. (2005). Baclofen-induced reduction of alcohol reinforcement in alcohol-preferring rats. *Alcohol* *36*: 161–8.
- Macey, D.J., Froestl, W., Koob, G.F., and Markou, A. (2001). Both GABA(B) receptor agonist and antagonists decreased brain stimulation reward in the rat. *Neuropharmacology* *40*: 676–85.
- MacLeod, B.A., Wang, J.T.C., Chung, C.C.W., Ries, C.R., Schwarz, S.K.W., and Puil, E. (2010). Analgesic properties of the novel amino acid, isovaline. *Anesth. Analg.* *110*: 1206–14.
- Maier, P.J., Marin, I., Grampp, T., Sommer, A., and Benke, D. (2010). Sustained glutamate receptor activation down-regulates GABAB receptors by shifting the balance from recycling to lysosomal degradation. *J. Biol. Chem.* *285*: 35606–35614.
- Margeta-Mitrovic, M., Jan, Y.N., and Jan, L.Y. (2001a). Function of GB1 and GB2 subunits in G protein coupling of GABA(B) receptors. *Proc. Natl. Acad. Sci. U. S. A.* *98*: 14649–14654.

Margeta-Mitrovic, M., Jan, Y.N., and Jan, L.Y. (2001b). Ligand-induced signal transduction within heterodimeric GABA(B) receptor. *Proc. Natl. Acad. Sci. U. S. A.* 98: 14643–14648.

Martel, P., Leo, D., Fulton, S., Bérard, M., and Trudeau, L.E. (2011). Role of Kv1 potassium channels in regulating dopamine release and presynaptic D2 receptor function. *PLoS One* 6: e20402.

Martin, S.C., Steiger, J.L., Gravielle, M.C., Lyons, H.R., Russek, S.J., and Farb, D.H. (2004). Differential expression of gamma-aminobutyric acid type B receptor subunit mRNAs in the developing nervous system and receptor coupling to adenylyl cyclase in embryonic neurons. *J. Comp. Neurol.* 473: 16–29.

Martí-Solano, M., Guixà-González, R., Sanz, F., Pastor, M., and Selent, J. (2013). Novel insights into biased agonism at G protein-coupled receptors and their potential for drug design. *Curr. Pharm. Des.* 19: 5156–66.

Mayfield, R.D., and Zahniser, N.R. (2001). Dopamine D2 receptor regulation of the dopamine transporter expressed in *Xenopus laevis* oocytes is voltage-independent. *Mol. Pharmacol.* 59: 113–21.

Meiergerd, S.M., Patterson, T. a, and Schenk, J.O. (1993). D2 receptors may modulate the function of the striatal transporter for dopamine: kinetic evidence from studies in vitro and in vivo. *J. Neurochem.* 61: 764–7.

Menon-Johansson, A.S., Berrow, N., and Dolphin, A.C. (1993). G(o) transduces GABAB-receptor modulation of N-type calcium channels in cultured dorsal root ganglion neurons. *Pflugers Arch.* 425: 335–343.

Metz, M., Gassmann, M., Fakler, B., Schaeren-Wiemers, N., and Bettler, B. (2011). Distribution of the auxiliary GABAB receptor subunits KCTD8, 12, 12b, and 16 in the mouse brain. *J. Comp. Neurol.* 519: 1435–1454.

Mintz, I.M., and Bean, B.P. (1993). GABAB receptor inhibition of P-type Ca<sup>2+</sup> channels in central neurons. *Neuron* 10: 889–898.

Missale, C., Nash, S.R., Robinson, S.W., Jaber, M., and Caron, M.G. (1998). Dopamine receptors: from structure to function. *Physiol. Rev.* 78: 189–225.

Molnár, T., Antal, K., Nyitrai, G., and Emri, Z. (2009). gamma-Hydroxybutyrate (GHB) induces GABA(B) receptor independent intracellular Ca<sup>2+</sup> transients in astrocytes, but has no effect on GHB or GABA(B) receptors of medium spiny neurons in the nucleus accumbens. *Neuroscience* 162: 268–81.

- Mombereau, C., Lhuillier, L., Kaupmann, K., and Cryan, J.F. (2007). GABAB receptor-positive modulation-induced blockade of the rewarding properties of nicotine is associated with a reduction in nucleus accumbens DeltaFosB accumulation. *J. Pharmacol. Exp. Ther.* 321: 172–7.
- Monsma, F.J., McVittie, L.D., Gerfen, C.R., Mahan, L.C., and Sibley, D.R. (1989). Multiple D2 dopamine receptors produced by alternative RNA splicing. *Nature* 342: 926–929.
- Morishita, R., Kato, K., and Asano, T. (1990). GABAB receptors couple to G proteins Go, Go\* and Gi1 but not to Gi2. *FEBS Lett.* 271: 231–235.
- Mutneja, M., Berton, F., Suen, K.F., Lüscher, C., and Slesinger, P.A. (2005). Endogenous RGS proteins enhance acute desensitization of GABAB receptor-activated GIRK currents in HEK-293T cells. *Pflugers Arch. Eur. J. Physiol.* 450: 61–73.
- Nakagawa, Y., Ishima, T., Ishibashi, Y., Tsuji, M., and Takashima, T. (1996). Involvement of GABAB receptor systems in experimental depression: baclofen but not bicuculline exacerbates helplessness in rats. *Brain Res.* 741: 240–5.
- Narayanaswami, V., Thompson, a C., Cassis, L. a, Bardo, M.T., and Dwoskin, L.P. (2013). Diet-induced obesity: dopamine transporter function, impulsivity and motivation. *Int. J. Obes. (Lond).* 37: 1095–103.
- Near, J.A., Bigelow, J.C., and Wightman, R.M. (1988). Comparison of uptake of dopamine in rat striatal chopped tissue and synaptosomes. *J. Pharmacol. Exp. Ther.* 245: 921–7.
- Negus, S.S., and Miller, L.L. (2014). Intracranial self-stimulation to evaluate abuse potential of drugs. *Pharmacol. Rev.* 66: 869–917.
- New, D.C., An, H., Ip, N.Y., and Wong, Y.H. (2006). GABAB heterodimeric receptors promote Ca<sup>2+</sup> influx via store-operated channels in rat cortical neurons and transfected chinese hamster ovary cells. *Neuroscience* 137: 1347–1358.
- Ng, T.K.Y., and Yung, K.K.L. (2001). Differential expression of GABABR1 and GABABR2 receptor immunoreactivity in neurochemically identified neurons of the rat neostriatum. *J. Comp. Neurol.* 433: 458–470.
- Nirenberg, M.J., Chan, J., Pohorille, a, Vaughan, R. a, Uhl, G.R., Kuhar, M.J., et al. (1997a). The dopamine transporter: comparative ultrastructure of dopaminergic axons in limbic and motor compartments of the nucleus accumbens. *J. Neurosci.* 17: 6899–907.
- Nirenberg, M.J., Chan, J., Vaughan, R.A., Uhl, G.R., Kuhar, M.J., and Pickel, V.M. (1997b). Immunogold localization of the dopamine transporter: an ultrastructural study of the rat ventral tegmental area. *J. Neurosci.* 17: 5255–62.

- Nirenberg, M.J., Vaughan, R.A., Uhl, G.R., Kuhar, M.J., and Pickel, V.M. (1996). The dopamine transporter is localized to dendritic and axonal plasma membranes of nigrostriatal dopaminergic neurons. *J. Neurosci.* *16*: 436–47.
- Nomura, R., Suzuki, Y., Kakizuka, A., and Jingami, H. (2008). Direct detection of the interaction between recombinant soluble extracellular regions in the heterodimeric metabotropic  $\gamma$ -aminobutyric acid receptor. *J. Biol. Chem.* *283*: 4665–4673.
- Oleson, E.B., Gentry, R.N., Chioma, V.C., and Cheer, J.F. (2012). Subsecond dopamine release in the nucleus accumbens predicts conditioned punishment and its successful avoidance. *J. Neurosci.* *32*: 14804–8.
- Onali, P., and Olanas, M.C. (1989). Involvement of adenylate cyclase inhibition in dopamine autoreceptor regulation of tyrosine hydroxylase in rat nucleus accumbens. *Neurosci. Lett.* *102*: 91–6.
- Ong, J., and Kerr, D.I. (1990). GABA-receptors in peripheral tissues. *Life Sci.* *46*: 1489–501.
- Orrù, A., Lai, P., Lobina, C., Maccioni, P., Piras, P., Scanu, L., et al. (2005). Reducing effect of the positive allosteric modulators of the GABAB receptor, CGP7930 and GS39783, on alcohol intake in alcohol-preferring rats. *Eur. J. Pharmacol.* *525*: 105–111.
- Ostrovskaya, O., Xie, K., Masuho, I., Fajardo-Serrano, A., Lujan, R., Wickman, K., et al. (2014). RGS7/G $\beta$ 5/R7BP complex regulates synaptic plasticity and memory by modulating hippocampal GABABR-GIRK signaling. *Elife* *3*: e02053.
- Overton, P., and Clark, D. (1992). Iontophoretically administered drugs acting at the N-methyl-D-aspartate receptor modulate burst firing in A9 dopamine neurons in the rat. *Synapse* *10*: 131–40.
- Overton, P.G., and Clark, D. (1997). Burst firing in midbrain dopaminergic neurons. *Brain Res. Brain Res. Rev.* *25*: 312–34.
- Padgett, C.L., Lalive, A.L., Tan, K.R., Terunuma, M., Munoz, M.B., Pangalos, M.N., et al. (2012). Methamphetamine-evoked depression of GABA(B) receptor signaling in GABA neurons of the VTA. *Neuron* *73*: 978–89.
- Padgett, C.L., and Slesinger, P.A. (2010). GABAB receptor coupling to G-proteins and ion channels. *Adv. Pharmacol.* *58*: 123–147.
- Pagano, A., Rovelli, G., Mosbacher, J., Lohmann, T., Duthey, B., Stauffer, D., et al. (2001). C-terminal interaction is essential for surface trafficking but not for heteromeric assembly of GABA(b) receptors. *J. Neurosci.* *21*: 1189–202.

Panagis, G., and Kastellakis, A. (2002). The effects of ventral tegmental administration of GABA(A), GABA(B), NMDA and AMPA receptor agonists on ventral pallidum self-stimulation. *Behav. Brain Res.* 131: 115–23.

Paquet, M., and Smith, Y. (2003). Group I metabotropic glutamate receptors in the monkey striatum: subsynaptic association with glutamatergic and dopaminergic afferents. *J. Neurosci.* 23: 7659–69.

Park, J., Aragona, B.J., Kile, B.M., Carelli, R.M., and Wightman, R.M. (2010). In vivo voltammetric monitoring of catecholamine release in subterritories of the nucleus accumbens shell. *Neuroscience* 169: 132–42.

Paterson, N.E., Froestl, W., and Markou, A. (2004). The GABAB receptor agonists baclofen and CGP44532 decreased nicotine self-administration in the rat. *Psychopharmacology (Berl)*. 172: 179–86.

Paterson, N.E., Froestl, W., and Markou, A. (2005). Repeated administration of the GABAB receptor agonist CGP44532 decreased nicotine self-administration, and acute administration decreased cue-induced reinstatement of nicotine-seeking in rats. *Neuropsychopharmacology* 30: 119–28.

Paterson, N.E., Vlachou, S., Guery, S., Kaupmann, K., Froestl, W., and Markou, A. (2008). Positive modulation of GABA(B) receptors decreased nicotine self-administration and counteracted nicotine-induced enhancement of brain reward function in rats. *J. Pharmacol. Exp. Ther.* 326: 306–14.

Peciña, S., Cagniard, B., Berridge, K.C., Aldridge, J.W., and Zhuang, X. (2003). Hyperdopaminergic mutant mice have higher ‘wanting’ but not ‘liking’ for sweet rewards. *J. Neurosci.* 23: 9395–402.

Perra, S., Clements, M. a, Bernier, B.E., and Morikawa, H. (2011). In vivo ethanol experience increases D(2) autoinhibition in the ventral tegmental area. *Neuropsychopharmacology* 36: 993–1002.

Perroy, J., Adam, L., Qanbar, R., Chénier, S., and Bouvier, M. (2003). Phosphorylation-independent desensitization of GABA(B) receptor by GRK4. *EMBO J.* 22: 3816–24.

Pfaff, T., Malitschek, B., Kaupmann, K., Prezeau, L., Pin, J.P., Bettler, B., et al. (1999). Alternative splicing generates a novel isoform of the rat metabotropic GABA(B)R1 receptor. *Eur. J. Neurosci.* 11: 2874–2882.

Phillips, P.E., and Stamford, J.A. (2000). Differential recruitment of N-, P- and Q-type voltage-operated calcium channels in striatal dopamine release evoked by ‘regular’ and ‘burst’ firing. *Brain Res.* 884: 139–46.

- Phillips, P.E.M., Stuber, G.D., Heien, M.L.A. V, Wightman, R.M., and Carelli, R.M. (2003). Subsecond dopamine release promotes cocaine seeking. *Nature* 422: 614–8.
- Pinard, A., Seddik, R., and Bettler, B. (2010). GABAB receptors: physiological functions and mechanisms of diversity. *Adv. Pharmacol.* 58: 231–55.
- Pitman, K.A., Puil, E., and Borgland, S.L. (2014). GABA B modulation of dopamine release in the nucleus accumbens core. *Eur. J. Neurosci.* 40: 3472–3480.
- Pontier, S.M., Lahaie, N., Ginham, R., St-Gelais, F., Bonin, H., Bell, D.J., et al. (2006). Coordinated action of NSF and PKC regulates GABAB receptor signaling efficacy. *EMBO J.* 25: 2698–2709.
- Pozza, M.F., Manuel, N.A., Steinmann, M., Froestl, W., and Davies, C.H. (1999). Comparison of antagonist potencies at pre- and post-synaptic GABA(B) receptors at inhibitory synapses in the CA1 region of the rat hippocampus. *Br. J. Pharmacol.* 127: 211–219.
- Prosser, H.M., Gill, C.H., Hirst, W.D., Grau, E., Robbins, M., Calver, A., et al. (2001). Epileptogenesis and enhanced prepulse inhibition in GABA(B1)-deficient mice. *Mol. Cell. Neurosci.* 17: 1059–1070.
- Puhl, M.D., Cason, A.M., Wojnicki, F.H.E., Corwin, R.L., and Grigson, P.S. (2011). A history of bingeing on fat enhances cocaine seeking and taking. *Behav. Neurosci.* 125: 930–942.
- Quéva, C., Bremner-Danielsen, M., Edlund, A., Ekstrand, A.J., Elg, S., Erickson, S., et al. (2003). Effects of GABA agonists on body temperature regulation in GABA(B1)-/- mice. *Br. J. Pharmacol.* 140: 315–322.
- Rajalu, M., Fritzius, T., Adelfinger, L., Jacquier, V., Besseyrias, V., Gassmann, M., et al. (2015). Pharmacological characterization of GABAB receptor subtypes assembled with auxiliary KCTD subunits. *Neuropharmacology* 88: 145–54.
- Ranaldi, R., and Poeggel, K. (2002). Baclofen decreases methamphetamine self-administration in rats. *Neuroreport* 13: 1107–10.
- Rebec, G.V, Christensen, J.R., Guerra, C., and Bardo, M.T. (1997). Regional and temporal differences in real-time dopamine efflux in the nucleus accumbens during free-choice novelty. *Brain Res.* 776: 61–7.
- Ren, X., and Mody, I. (2003). Gamma-hydroxybutyrate reduces mitogen-activated protein kinase phosphorylation via GABA B receptor activation in mouse frontal cortex and hippocampus. *J. Biol. Chem.* 278: 42006–42011.

- Ren, X., and Mody, I. (2006).  $\gamma$ -Hydroxybutyrate induces cyclic amp-responsive element-binding protein phosphorylation in mouse hippocampus: An involvement of GABAB receptors and cAMP-dependent protein kinase activation. *Neuroscience* 141: 269–275.
- Rice, M.E., and Cragg, S.J. (2004). Nicotine amplifies reward-related dopamine signals in striatum. *Nat. Neurosci.* 7: 583–4.
- Rickhag, M., Hansen, F.H., Sørensen, G., Strandfelt, K.N., Andresen, B., Gotfryd, K., et al. (2013). A C-terminal PDZ domain-binding sequence is required for striatal distribution of the dopamine transporter. *Nat. Commun.* 4: 1580.
- Ritz, M.C., Lamb, R.J., Goldberg, S.R., and Kuhar, M.J. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* 237: 1219–23.
- Robbins, M.J., Calver, A.R., Filippov, A.K., Hirst, W.D., Russell, R.B., Wood, M.D., et al. (2001). GABA(B2) is essential for g-protein coupling of the GABA(B) receptor heterodimer. *J. Neurosci.* 21: 8043–8052.
- Roberts, D.C., and Andrews, M.M. (1997). Baclofen suppression of cocaine self-administration: demonstration using a discrete trials procedure. *Psychopharmacology (Berl)*. 131: 271–7.
- Roberts, D.C., Andrews, M.M., and Vickers, G.J. (1996). Baclofen attenuates the reinforcing effects of cocaine in rats. *Neuropsychopharmacology* 15: 417–23.
- Robertson, B., and Taylor, W.R. (1986). Effects of gamma-aminobutyric acid and (-)-baclofen on calcium and potassium currents in cat dorsal root ganglion neurones in vitro. *Br. J. Pharmacol.* 89: 661–672.
- Robinson, T.E., and Berridge, K.C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res. Brain Res. Rev.* 18: 247–91.
- Roitman, M.F. (2004). Dopamine Operates as a Subsecond Modulator of Food Seeking. *J. Neurosci.* 24: 1265–1271.
- Rolls, B.J., Rowe, E.A., and Turner, R.C. (1980). Persistent obesity in rats following a period of consumption of a mixed, high energy diet. *J. Physiol.* 298: 415–27.
- Rothwell, N.J., and Stock, M.J. (1981). Regulation of energy balance. *Annu. Rev. Nutr.* 1: 235–56.
- Rubinstein, M., Peleg, S., Berlin, S., Brass, D., and Dascal, N. (2007). Galphai3 primes the G protein-activated K<sup>+</sup> channels for activation by coexpressed Gbetagamma in intact *Xenopus* oocytes. *J. Physiol.* 581: 17–32.

Sadja, R., Alagem, N., and Reuveny, E. (2003). Gating of GIRK channels: Details of an intricate, membrane-delimited signaling complex. *Neuron* 39: 9–12.

Sahraei, H., Etemadi, L., Rostami, P., Pourmotabbed, A., Zarrindast, M.-R., Shams, J., et al. (2009). GABAB receptors within the ventral tegmental area are involved in the expression and acquisition of morphine-induced place preference in morphine-sensitized rats. *Pharmacol. Biochem. Behav.* 91: 409–16.

Saint, D.A., Thomas, T., and Gage, P.W. (1990). GABAB agonists modulate a transient potassium current in cultured mammalian hippocampal neurons. *Neurosci. Lett.* 118: 9–13.

Sakaba, T., and Neher, E. (2003). Direct modulation of synaptic vesicle priming by GABA(B) receptor activation at a glutamatergic synapse. *Nature* 424: 775–778.

Salah, R.S., Kuhn, D.M., and Galloway, M.P. (1989). Dopamine autoreceptors modulate the phosphorylation of tyrosine hydroxylase in rat striatal slices. *J. Neurochem.* 52: 1517–22.

Salamone, J.D., Zigmond, M.J., and Stricker, E.M. (1990). Characterization of the impaired feeding behavior in rats given haloperidol or dopamine-depleting brain lesions. *Neuroscience* 39: 17–24.

Salminen, O., Murphy, K.L., McIntosh, J.M., Drago, J., Marks, M.J., Collins, A.C., et al. (2004). Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. *Mol. Pharmacol.* 65: 1526–35.

Sase, A., Brückner, H., Höger, H., Pollak, A., and Lubec, G. (2013). Behavioral effects of alpha-alkylated amino acid analogs in the C57BL/6J mouse. *Behav. Brain Res.* 252: 432–8.

Saunders, B.T., Yager, L.M., and Robinson, T.E. (2013). Cue-evoked cocaine ‘craving’: role of dopamine in the accumbens core. *J. Neurosci.* 33: 13989–4000.

Sawa, G.M., and Paty, D.W. (1979). The use of baclofen in treatment of spasticity in multiple sclerosis.

Scanziani, M. (2000). GABA spillover activates postsynaptic GABA(B) receptors to control rhythmic hippocampal activity. *Neuron* 25: 673–681.

Schmitz, Y., Benoit-Marand, M., Gonon, F., and Sulzer, D. (2003). Presynaptic regulation of dopaminergic neurotransmission. *J. Neurochem.* 87: 273–89.

Schmitz, Y., Schmauss, C., and Sulzer, D. (2002). Altered dopamine release and uptake kinetics in mice lacking D2 receptors. *J. Neurosci.* 22: 8002–9.

Schuler, V., Lüscher, C., Blanchet, C., Klix, N., Sansig, G., Klebs, K., et al. (2001). Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic GABAB responses in mice lacking GABAB(1). *Neuron* 31: 47–58.

Schwenk, J., Metz, M., Zolles, G., Turecek, R., Fritzius, T., Bildl, W., et al. (2010). Native GABA(B) receptors are heteromultimers with a family of auxiliary subunits. *Nature* 465: 231–5.

Seddik, R., Jungblut, S.P., Silander, O.K., Rajalu, M., Fritzius, T., Besseyrias, V., et al. (2012). Opposite effects of KCTD subunit domains on GABA(B) receptor-mediated desensitization. *J. Biol. Chem.* 287: 39869–39877.

Sharma, S., and Fulton, S. (2013). Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry. *Int. J. Obes. (Lond.)* 37: 382–9.

Shiba, K., Mori, H., and Hisada, K. (1989). Analogues of alpha-aminoisobutyric acid with various alkyl groups as potential tumour imaging agents; effects of chain length on tumour and normal tissue specificity. *Nucl. Med. Commun.* 10: 751–8.

Shin, A.C., Townsend, R.L., Patterson, L.M., and Berthoud, H.R. (2011a). ‘Liking’ and ‘wanting’ of sweet and oily food stimuli as affected by high-fat diet-induced obesity, weight loss, leptin, and genetic predisposition. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 301: R1267–80.

Shin, D.S., Yu, W., Sutton, A., Calos, M., Puil, E., and Carlen, P.L. (2011b). Isovaline, a rare amino acid, has anticonvulsant properties in two in vitro hippocampal seizure models by increasing interneuronal activity. *Epilepsia* 52: 2084–93.

Shoaib, M., Swanner, L.S., Beyer, C.E., Goldberg, S.R., and Schindler, C.W. (1998). The GABAB agonist baclofen modifies cocaine self-administration in rats. *Behav. Pharmacol.* 9: 195–206.

Shoptaw, S., Yang, X., Rotheram-Fuller, E.J., Hsieh, Y.M., Kintaudi, P.C., Charuvastra, V.C., et al. (2003). Randomized placebo-controlled trial of baclofen for cocaine dependence: preliminary effects for individuals with chronic patterns of cocaine use. *J. Clin. Psychiatry* 64: 1440–8.

Simon, G.E., Korff, M. Von, Saunders, K., Miglioretti, D.L., Crane, P.K., van Belle, G., et al. (2006). Association between obesity and psychiatric disorders in the US adult population. *Arch. Gen. Psychiatry* 63: 824–30.

Slattery, D. A., Markou, A., Froestl, W., and Cryan, J.F. (2005). The GABAB receptor-positive modulator GS39783 and the GABAB receptor agonist baclofen attenuate the reward-facilitating effects of cocaine: intracranial self-stimulation studies in the rat. *Neuropsychopharmacology* 30: 2065–2072.

- Slesinger, P.A., Stoffel, M., Jan, Y.N., and Jan, L.Y. (1997). Defective gamma-aminobutyric acid type B receptor-activated inwardly rectifying K<sup>+</sup> currents in cerebellar granule cells isolated from weaver and Girk2 null mutant mice. *Proc. Natl. Acad. Sci. U. S. A.* 94: 12210–12217.
- Smith, M.A., Yancey, D.L., Morgan, D., Liu, Y., Froestl, W., and Roberts, D.C.S. (2004). Effects of positive allosteric modulators of the GABA B receptor on cocaine self-administration in rats. *Psychopharmacology (Berl)*. 173: 105–111.
- Smolders, I., Klippel, N. De, Sarre, S., Ebinger, G., and Michotte, Y. (1995). Tonic GABA-ergic modulation of striatal dopamine release studied by in vivo microdialysis in the freely moving rat. *Eur. J. Pharmacol.* 284: 83–91.
- Sodickson, D.L., and Bean, B.P. (1996). GABAB receptor-activated inwardly rectifying potassium current in dissociated hippocampal CA3 neurons. *J. Neurosci.* 16: 6374–85.
- South, T., and Huang, X.F. (2008). High-fat diet exposure increases dopamine D2 receptor and decreases dopamine transporter receptor binding density in the nucleus accumbens and caudate putamen of mice. *Neurochem. Res.* 33: 598–605.
- Spano, P.F., Govoni, S., and Trabucchi, M. (1978). Studies on the pharmacological properties of dopamine receptors in various areas of the central nervous system. *Adv. Biochem. Psychopharmacol.* 19: 155–65.
- Speed, N., Saunders, C., Davis, A.R., Owens, W.A., Matthies, H.J.G., Saadat, S., et al. (2011). Impaired striatal Akt signaling disrupts dopamine homeostasis and increases feeding. *PLoS One* 6: e25169.
- Stadel, J.M., Shorr, R.G., Limbird, L.E., and Lefkowitz, R.J. (1981). Evidence that a beta-adrenergic receptor-associated guanine nucleotide regulatory protein conveys guanosine 5'-O-(3-thiotriphosphate)-dependent adenylate cyclase activity. *J. Biol. Chem.* 256: 8718–8723.
- Steiger, J.L., Bandyopadhyay, S., Farb, D.H., and Russek, S.J. (2004). cAMP response element-binding protein, activating transcription factor-4, and upstream stimulatory factor differentially control hippocampal GABABR1a and GABABR1b subunit gene expression through alternative promoters. *J. Neurosci.* 24: 6115–6126.
- Steketee, J.D., and Kalivas, P.W. (2011). Drug wanting: behavioral sensitization and relapse to drug-seeking behavior. *Pharmacol. Rev.* 63: 348–65.
- Stice, E., Spoor, S., Bohon, C., and Small, D.M. (2008a). Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. *Science* 322: 449–52.

Stice, E., Spoor, S., Bohon, C., Veldhuizen, M.G., and Small, D.M. (2008b). Relation of reward from food intake and anticipated food intake to obesity: a functional magnetic resonance imaging study. *J. Abnorm. Psychol.* *117*: 924–35.

Straub, C., Tritsch, N.X., Hagan, N.A., Gu, C., and Sabatini, B.L. (2014). Multiphasic modulation of cholinergic interneurons by nigrostriatal afferents. *J. Neurosci.* *34*: 8557–69.

Sugam, J. A., Day, J.J., Wightman, R.M., and Carelli, R.M. (2012). Phasic nucleus accumbens dopamine encodes risk-based decision-making behavior. *Biol. Psychiatry* *71*: 199–205.

Sugita, S., Johnson, S.W., and North, R.A. (1992). Synaptic inputs to GABAA and GABAB receptors originate from discrete afferent neurons. *Neurosci. Lett.* *134*: 207–11.

Sulzer, D., Chen, T.K., Lau, Y.Y., Kristensen, H., Rayport, S., and Ewing, A. (1995). Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. *J. Neurosci.* *15*: 4102–8.

Surmeier, D.J., Shen, W., Day, M., Gertler, T., Chan, S., Tian, X., et al. (2010). The role of dopamine in modulating the structure and function of striatal circuits. *Prog. Brain Res.* *183*: 149–67.

Swanson, L.W. (1982). The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Res. Bull.* *9*: 321–353.

Swillens, S., Juvent, M., and Dumont, J.E. (1979). Slow GDP dissociation from the guanyl nucleotide-binding site of turkey erythrocyte membranes as the limiting step in the activation of adenylate cyclase by beta-adrenergic agonists. *FEBS Lett.* *108*: 365–8.

Taniyama, K., Takeda, K., Ando, H., and Tanaka, C. (1991). Expression of the GABAB receptor in *Xenopus* oocytes and desensitization by activation of protein kinase C. *Adv. Exp. Med. Biol.* *287*: 413–20.

Tepper, J.M., Martin, L.P., and Anderson, D.R. (1995). GABAA receptor-mediated inhibition of rat substantia nigra dopaminergic neurons by pars reticulata projection neurons. *J. Neurosci.* *15*: 3092–103.

Terunuma, M., Vargas, K.J., Wilkins, M.E., Ramírez, O.A., Jaureguiberry-Bravo, M., Pangalos, M.N., et al. (2010). Prolonged activation of NMDA receptors promotes dephosphorylation and alters postendocytic sorting of GABAB receptors. *Proc. Natl. Acad. Sci. U. S. A.* *107*: 13918–13923.

Threlfell, S., Clements, M. A., Khodai, T., Pienaar, I.S., Exley, R., Wess, J., et al. (2010). Striatal muscarinic receptors promote activity dependence of dopamine transmission via distinct

receptor subtypes on cholinergic interneurons in ventral versus dorsal striatum. *J. Neurosci.* *30*: 3398–408.

Thuault, S.J., Brown, J.T., Sheardown, S.A., Jourdain, S., Fairfax, B., Spencer, J.P., et al. (2004). The GABA(B2) subunit is critical for the trafficking and function of native GABA(B) receptors. *Biochem. Pharmacol.* *68*: 1655–66.

Tiao, J.Y., Bradaia, A., Biermann, B., Kaupmann, K., Metz, M., Haller, C., et al. (2008). The sushi domains of secreted GABAB1 isoforms selectively impair GABAB heteroreceptor function. *J. Biol. Chem.* *283*: 31005–31011.

Tsuji, M., Nakagawa, Y., Ishibashi, Y., Yoshii, T., Takashima, T., Shimada, M., et al. (1996). Activation of ventral tegmental GABAB receptors inhibits morphine-induced place preference in rats. *Eur. J. Pharmacol.* *313*: 169–73.

Tu, H., Rondard, P., Xu, C., Bertaso, F., Cao, F., Zhang, X., et al. (2007). Dominant role of GABAB2 and Gbetagamma for GABAB receptor-mediated-ERK1/2/CREB pathway in cerebellar neurons. *Cell. Signal.* *19*: 1996–2002.

Turecek, R., Schwenk, J., Fritzius, T., Ivankova, K., Zolles, G., Adelfinger, L., et al. (2014). Auxiliary GABAB Receptor Subunits Uncouple G Protein  $\beta\gamma$  Subunits from Effector Channels to Induce Desensitization. *Neuron* *82*: 1032–44.

Tyacke, R.J., Lingford-Hughes, A., Reed, L.J., and Nutt, D.J. (2010). GABAB receptors in addiction and its treatment. *Adv. Pharmacol.* *58*: 373–96.

Uchimura, N., and North, R.A. (1991). Baclofen and adenosine inhibit synaptic potentials mediated by gamma-aminobutyric acid and glutamate release in rat nucleus accumbens. *J. Pharmacol. Exp. Ther.* *258*: 663–8.

Uezono, Y., Akihara, M., Kaibara, M., Kawano, C., Shibuya, I., Ueda, Y., et al. (1998). Activation of inwardly rectifying K<sup>+</sup> channels by GABA-B receptors expressed in *Xenopus* oocytes. *Neuroreport* *9*: 583–587.

Urwyler, S., Gjoni, T., Koljatić, J., and Dupuis, D.S. (2005). Mechanisms of allosteric modulation at GABAB receptors by CGP7930 and GS39783: Effects on affinities and efficacies of orthosteric ligands with distinct intrinsic properties. *Neuropharmacology* *48*: 343–353.

Urwyler, S., Mosbacher, J., Lingenhoebl, K., Heid, J., Hofstetter, K., Froestl, W., et al. (2001). Positive allosteric modulation of native and recombinant gamma-aminobutyric acid(B) receptors by 2,6-Di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Mol. Pharmacol.* *60*: 963–971.

- Urwyler, S., Pozza, M.F., Lingenhoehl, K., Mosbacher, J., Lampert, C., Froestl, W., et al. (2003). N,N'-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and structurally related compounds: novel allosteric enhancers of gamma-aminobutyric acidB receptor function. *J. Pharmacol. Exp. Ther.* 307: 322–330.
- Vargas, K.J., Terunuma, M., Tello, J.A., Pangalos, M.N., Moss, S.J., and Couve, A. (2008). The availability of surface GABAB receptors is independent of  $\gamma$ -aminobutyric acid but controlled by glutamate in central neurons. *J. Biol. Chem.* 283: 24641–24648.
- van Bree, J.B., Audus, K.L., and Borchardt, R.T. (1988). Carrier-mediated transport of baclofen across monolayers of bovine brain endothelial cells in primary culture. *Pharm. Res.* 5: 369–371.
- van Bree, J.B., Heijligers-Feijen, C.D., Boer, A.G. De, Danhof, M., and Breimer, D.D. (1991). Stereoselective transport of baclofen across the blood-brain barrier in rats as determined by the united impulse response methodology. *Pharm. Res.* 8: 259–262.
- van de Giessen, E., la Fleur, S.E., de Bruin, K., van den Brink, W., and Booij, J. (2012). Free-choice and no-choice high-fat diets affect striatal dopamine D2/3 receptor availability, caloric intake, and adiposity. *Obesity (Silver Spring)*. 20: 1738–40.
- van de Giessen, E., la Fleur, S.E., Eggels, L., de Bruin, K., van den Brink, W., and Booij, J. (2013). High fat/carbohydrate ratio but not total energy intake induces lower striatal dopamine D2/3 receptor availability in diet-induced obesity. *Int. J. Obes. (Lond)*. 37: 754–7.
- van Nieuwenhuijzen, P.S., and McGregor, I.S. (2009). Sedative and hypothermic effects of gamma-hydroxybutyrate (GHB) in rats alone and in combination with other drugs: assessment using biotelemetry. *Drug Alcohol Depend.* 103: 137–47.
- Velez-Fort, M., Audinat, E., and Angulo, M.C. (2012). Central Role of GABA in Neuron-Glia Interactions. *Neurosci.* 18: 237–250.
- Venton, B.J., Zhang, H., Garris, P.A., Phillips, P.E.M., Sulzer, D., and Wightman, R.M. (2003). Real-time decoding of dopamine concentration changes in the caudate-putamen during tonic and phasic firing. *J. Neurochem.* 87: 1284–95.
- Vigot, R., Barbieri, S., Bräuner-Osborne, H., Turecek, R., Shigemoto, R., Zhang, Y.P., et al. (2006). Differential Compartmentalization and Distinct Functions of GABAB Receptor Variants. *Neuron* 50: 589–601.
- Vlachou, S., and Markou, A. (2010). GABAB receptors in reward processes. *Adv. Pharmacol.* 58: 315–71.

- Waldmeier, P.C., Wicki, P., Feldtrauer, J.J., Mickel, S.J., Bittiger, H., and Baumann, P.A. (1994). GABA and glutamate release affected by GABAB receptor antagonists with similar potency: no evidence for pharmacologically different presynaptic receptors. *Br. J. Pharmacol.* *113*: 1515–21.
- Walker, B.M., and Koob, G.F. (2007). The gamma-aminobutyric acid-B receptor agonist baclofen attenuates responding for ethanol in ethanol-dependent rats. *Alcohol. Clin. Exp. Res.* *31*: 11–8.
- Wang, G.J., Volkow, N.D., Logan, J., Pappas, N.R., Wong, C.T., Zhu, W., et al. (2001). Brain dopamine and obesity. *Lancet* *357*: 354–357.
- Warren, M., Frost-Pineda, K., and Gold, M. (2005). Body mass index and marijuana use. *J. Addict. Dis.* *24*: 95–100.
- Watson, N., Linder, M.E., Druey, K.M., Kehrl, J.H., and Blumer, K.J. (1996). RGS family members: GTPase-activating proteins for heterotrimeric G-protein alpha-subunits. *Nature* *383*: 172–175.
- Wei, K., Eubanks, J.H., Francis, J., Jia, Z., and Snead, O.C. (2001a). Cloning and tissue distribution of a novel isoform of the rat GABA(B)R1 receptor subunit. *Neuroreport* *12*: 833–837.
- Wei, K., Jia, Z., Wang, Y.T., Yang, J., Liu, C.C., and Snead, O.C. (2001b). Cloning and characterization of a novel variant of rat GABA(B)R1 with a truncated C-terminus. *Brain Res. Mol. Brain Res.* *89*: 103–110.
- Westerink, B.H., Kwint, H.F., and deVries, J.B. (1996). The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. *J. Neurosci.* *16*: 2605–11.
- White, J.H., McIlhinney, R.A., Wise, A., Ciruela, F., Chan, W.Y., Emson, P.C., et al. (2000). The GABAB receptor interacts directly with the related transcription factors CREB2 and ATFx. *Proc. Natl. Acad. Sci. U. S. A.* *97*: 13967–13972.
- White, J.H., Wise, A., Main, M.J., Green, A., Fraser, N.J., Disney, G.H., et al. (1998). Heterodimerization is required for the formation of a functional GABA(B) receptor. *Nature* *396*: 679–82.
- Whitehead, R.A., Puil, E., Ries, C.R., Schwarz, S.K.W., Wall, R.A., Cooke, J.E., et al. (2012). GABA(B) receptor-mediated selective peripheral analgesia by the non-proteinogenic amino acid, isovaline. *Neuroscience* *213*: 154–60.
- Wightman, R.M., Amatore, C., Engstrom, R.C., Hale, P.D., Kristensen, E.W., Kuhr, W.G., et al. (1988). Real-time characterization of dopamine overflow and uptake in the rat striatum. *Neuroscience* *25*: 513–23.

- Wightman, R.M., and Zimmerman, J.B. (1990). Control of dopamine extracellular concentration in rat striatum by impulse flow and uptake. *Brain Res. Brain Res. Rev.* 15: 135–144.
- Wilkins, M.E., Li, X., and Smart, T.G. (2008). Tracking cell surface GABAB receptors using an alpha-bungarotoxin tag. *J. Biol. Chem.* 283: 34745–34752.
- Willars, G.B., and Challiss, R.A.J. (2004). *Receptor Signal Transduction Protocols* (New Jersey: Humana Press).
- Willick, M.L., and Kokkinidis, L. (1995). The effects of ventral tegmental administration of GABAA, GABAB and NMDA receptor agonists on medial forebrain bundle self-stimulation. *Behav. Brain Res.* 70: 31–6.
- Wise, R.A., and Bozarth, M.A. (1987). A psychomotor stimulant theory of addiction. *Psychol. Rev.* 94: 469–92.
- Wit, H. De, and Wise, R.A. (1977). Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide, but not with the noradrenergic blockers phentolamine or phenoxybenzamine. *Can. J. Psychol.* 31: 195–203.
- Wojnicki, F.H.E., Roberts, D.C.S., and Corwin, R.L.W. (2006). Effects of baclofen on operant performance for food pellets and vegetable shortening after a history of binge-type behavior in non-food deprived rats. *Pharmacol. Biochem. Behav.* 84: 197–206.
- Wong, K.J., Wojnicki, F.H.W., and Corwin, R.L.W. (2009). Baclofen, raclopride, and naltrexone differentially affect intake of fat/sucrose mixtures under limited access conditions. *Pharmacol. Biochem. Behav.* 92: 528–536.
- Wydra, K., Golembiowska, K., Zaniewska, M., Kamińska, K., Ferraro, L., Fuxe, K., et al. (2013). Accumbal and pallidal dopamine, glutamate and GABA overflow during cocaine self-administration and its extinction in rats. *Addict. Biol.* 18: 307–24.
- Xi, Z.X., Ramamoorthy, S., Shen, H., Lake, R., Samuvel, D.J., and Kalivas, P.W. (2003). GABA transmission in the nucleus accumbens is altered after withdrawal from repeated cocaine. *J. Neurosci.* 23: 3498–505.
- Xi, Z.X., and Stein, E. A. (1999). Baclofen inhibits heroin self-administration behavior and mesolimbic dopamine release. *J. Pharmacol. Exp. Ther.* 290: 1369–74.
- Xi, Z.X., and Stein, E.A. (1998). Nucleus accumbens dopamine release modulation by mesolimbic GABAA receptors-an in vivo electrochemical study. *Brain Res.* 798: 156–65.

- Xie, C., Markesbery, W.R., and Lovell, M.A. (2000). Survival of hippocampal and cortical neurons in a mixture of MEM+ and B27-supplemented neurobasal medium. *Free Radic. Biol. Med.* 28: 665–72.
- Xie, K., Allen, K.L., Kourrich, S., Colón-Saez, J., Thomas, M.J., Wickman, K., et al. (2010). Gbeta5 recruits R7 RGS proteins to GIRK channels to regulate the timing of neuronal inhibitory signaling. *Nat. Neurosci.* 13: 661–3.
- Xu, J., and Wojcik, W.J. (1986). Gamma aminobutyric acid B receptor-mediated inhibition of adenylate cyclase in cultured cerebellar granule cells: blockade by islet-activating protein. *J. Pharmacol. Exp. Ther.* 239: 568–73.
- Yamada, J., Saitow, F., Satake, S., Kiyohara, T., and Konishi, S. (1999). GABA(B) receptor-mediated presynaptic inhibition of glutamatergic and GABAergic transmission in the basolateral amygdala. *Neuropharmacology* 38: 1743–1753.
- Yokel, R.A., and Wise, R.A. (1978). Amphetamine- type reinforcement by dopaminergic agonists in the rat. *Psychopharmacology (Berl)*. 58: 289–96.
- Yokoo, H., Goldstein, M., and Meller, E. (1988). Receptor reserve at striatal dopamine receptors modulating the release of [3H]dopamine. *Eur. J. Pharmacol.* 155: 323–7.
- Yorgason, J.T., España, R. A., and Jones, S.R. (2011). Demon voltammetry and analysis software: analysis of cocaine-induced alterations in dopamine signaling using multiple kinetic measures. *J. Neurosci. Methods* 202: 158–64.
- Yoshida, M., Yokoo, H., Tanaka, T., Emoto, H., and Tanaka, M. (1994). Opposite changes in the mesolimbic dopamine metabolism in the nerve terminal and cell body sites induced by locally infused baclofen in the rat. *Brain Res.* 636: 111–4.
- Young, K.A., Franklin, T.R., Roberts, D.C.S., Jagannathan, K., Suh, J.J., Wetherill, R.R., et al. (2014). Nipping Cue Reactivity in the Bud: Baclofen Prevents Limbic Activation Elicited by Subliminal Drug Cues. *J. Neurosci.* 34: 5038–5043.
- Yu, B., Yamada, K., and Gallagher, J.P. (1999). GABA(B) auto- versus hetero-receptor sensitivity: Implications for novel pharmacotherapy. *Neuropharmacology* 38: 1805–1809.
- Yu, W., Smith, A.B., Pilitsis, J., and Shin, D.S. (2014). Isovaline attenuates epileptiform activity and seizure behavior in 4-aminopyridine treated rats. *Epilepsy Res.* 108: 331–5.
- Zarrindast, M.R., Hosseini-Nia, T., and Allah-Maddadi, S. (1989). Food intake suppressant effect of baclofen in rats. *Gen. Pharmacol.* 20: 701–3.

- Zhang, H., and Sulzer, D. (2003). Glutamate spillover in the striatum depresses dopaminergic transmission by activating group I metabotropic glutamate receptors. *J. Neurosci.* 23: 10585–92.
- Zhang, H., and Sulzer, D. (2004). Frequency-dependent modulation of dopamine release by nicotine. *Nat. Neurosci.* 7: 581–2.
- Zhang, H., and Sulzer, D. (2012). Regulation of striatal dopamine release by presynaptic auto- and heteroreceptors. *Basal Ganglia* 2: 5–13.
- Zhang, L., Doyon, W.M., Clark, J.J., Phillips, P.E.M., and Dani, J.A. (2009a). Controls of tonic and phasic dopamine transmission in the dorsal and ventral striatum. *Mol. Pharmacol.* 76: 396–404.
- Zhang, T., Zhang, L., Liang, Y., Siapas, A.G., Zhou, F.-M., and Dani, J. A. (2009b). Dopamine signaling differences in the nucleus accumbens and dorsal striatum exploited by nicotine. *J. Neurosci.* 29: 4035–43.
- Zhang, W., Yamada, M., Gomeza, J., Basile, A.S., and Wess, J. (2002). Multiple muscarinic acetylcholine receptor subtypes modulate striatal dopamine release, as studied with M1-M5 muscarinic receptor knock-out mice. *J. Neurosci.* 22: 6347–52.
- Zhou, F.M., Liang, Y., and Dani, J. A. (2001). Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. *Nat. Neurosci.* 4: 1224–9.
- Zilberter, Y., Kaiser, K.M., and Sakmann, B. (1999). Dendritic GABA release depresses excitatory transmission between layer 2/3 pyramidal and bitufted neurons in rat neocortex. *Neuron* 24: 979–988.
- Zoli, M., Moretti, M., Zanardi, A., McIntosh, J.M., Clementi, F., and Gotti, C. (2002). Identification of the nicotinic receptor subtypes expressed on dopaminergic terminals in the rat striatum. *J. Neurosci.* 22: 8785–9.
- Zweifel, L.S., Parker, J.G., Lobb, C.J., Rainwater, A., Wall, V.Z., Fadok, J.P., et al. (2009). Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior. *Proc. Natl. Acad. Sci. U. S. A.* 106: 7281–8.