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TITLE: Central and Peripheral Mechanisms of Antipsychotic Medication-Induced Metabolic Dysregulation

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<b>14. ABSTRACT</b> Antipsychotic drugs (APDs) are widely used psychotropic medications, though they have significant metabolic side effects. While the mechanisms for these metabolic disturbances are poorly understood, the single known unifying property of all APDs is their blockade of the dopamine D <sub>2</sub> (D2R) and D <sub>3</sub> (D3R) receptors. We therefore hypothesize that D2R and/or D3R mediate the metabolic side effects of APDs both centrally in the hypothalamus and peripherally in pancreas, areas critical for metabolic regulation. In Year 1 of this award, we have completed the design of a D3R-flox mouse in order to selectively knock out expression of D3R in the hypothalamus and pancreatic beta cells. The resulting transgenic mice are being tested to confirm the successful production of the strain. In parallel, we have completed construction of novel inducible transgenic hypothalamic- and pancreatic beta cell-specific D2R knockout (KO) mice. Additionally, using pancreatic islets isolated from beta cell-selective D2R KO mice and complete D3R KO mice, we found diminished inhibition of stimulated insulin secretion in both strains relative to littermate controls, suggesting a role for both receptors in mediating insulin secretion.					
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## 1. INTRODUCTION

Antipsychotic drugs (APDs) are widely used psychotropic medications for numerous psychiatric illnesses including schizophrenia, posttraumatic stress disorder and depression. However, these medications also have significant metabolic side effects characterized by substantial weight gain, glucose intolerance, insulin resistance, hypertension and dyslipidemia as well as increased risks for type 2 diabetes and cardiovascular disease. Indeed, the prevalence of these APD-induced metabolic side effects in Veterans is more than twice that of the general population. However, the mechanisms for these metabolic disturbances are not well understood. Significantly, all APDs cause these side effects to differing degrees and ultimately result in life-shortening morbidity. A potentially important clue is that the single known unifying property of all APDs is their blockade of the dopamine D<sub>2</sub> (D2R) and D<sub>3</sub> (D3R) receptors, suggesting a role for these receptors in APD metabolic side effects. Consistent with this, D2R and D3R are expressed both centrally in the hypothalamus in regions mediating appetite and feeding behavior as well as peripherally in insulin-releasing pancreatic beta cells, key regulators of metabolism. We previously showed that activation of pancreatic beta cell D2R and D3R inhibited glucose-stimulated insulin secretion (GSIS) and that APD-induced receptor inhibition disrupted this regulatory mechanism. Thus, our central hypothesis is that D2R and/or D3R are critical regulators of metabolism and mediate the metabolic side effects of APDs both centrally in the hypothalamus and peripherally in pancreas. However, the relative contributions of peripheral and central D2R and D3R to APD-induced metabolic dysregulation are unknown. To disentangle these mechanisms, in partnership with Partnering PI Dr. Gary Schwartz, we will aim to do the following: (1) to identify contributions of hypothalamic D2R and D3R action in APD-induced weight gain and metabolic dysregulation *in vivo*; (2) to identify the relationship of peripheral D2R and D3R to APD-induced weight gain and metabolic dysfunction *in vivo*; and (3) to identify APD-mediated effects on insulin and DA release in pancreatic beta cells using real-time imaging. Key to these aims is the generation of tissue-specific D2R and D3R knockout (KO) mice targeting either hypothalamus or pancreatic beta cells. Moreover, in focusing on the peripheral contributions of pancreatic D2R and D3R, we have also developed new and highly sensitive optical and biochemical assays to study D2R- and D3R-mediated effects on insulin and DA release in real-time. We have applied these new assays to an experimentally tractable model using the well-characterized rat beta cell-derived INS-1E cell line for our *in vitro* studies, in addition to our work in the D2R and D3R KO pancreatic islets. In the short term, our work will elucidate the anatomical and functional mechanisms of APD-induced metabolic side effects. In the longer term, we will use our findings to develop better-targeted APDs that can selectively reverse these drugs' metabolic side effects while preserving their clinical efficacy.

## 2. KEYWORDS

Keywords relevant to the work proposed here include:

1. Antipsychotic drug (APD)
2. Dopamine (DA)
3. Dopamine D<sub>2</sub> Receptor (D2R)
4. Dopamine D<sub>3</sub> Receptor (D3R)
5. Insulin
6. Glucose-stimulated insulin secretion (GSIS)
7. Diabetes
8. Metabolism

## 3. ACCOMPLISHMENTS

### • **What were the major goals of the project?**

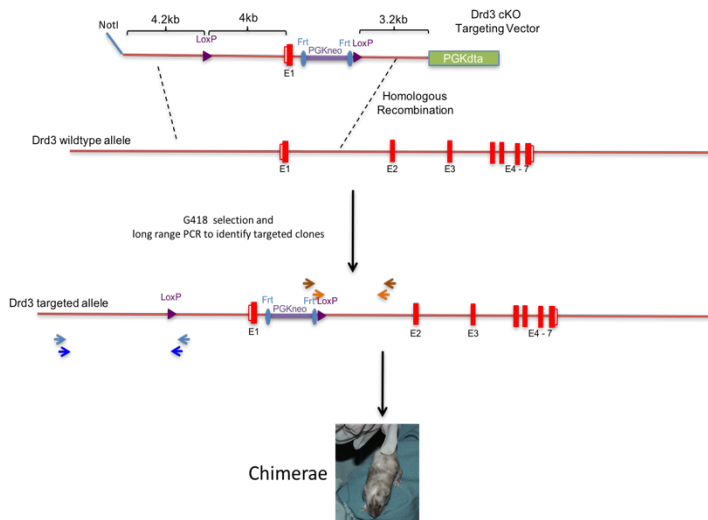
The major goals of the project as stated in the approved SOW are as follows:

- A. Metabolic characterization of hypothalamus-specific D2R and D3R knockout mice in the presence or absence of APD treatment
- B. Metabolic characterization of pancreatic beta cell-specific D2R and D3R knockout mice in the presence or absence of APD treatment

- C. Treatment with domperidone to determine whether peripheral D2R/D3R blockade alone can produce relevant metabolic disease
- D. Determine the precise contributions of D2R and D3R to glucose-stimulated insulin and dopamine release using pancreatic islets from pancreatic beta cell-selective D2R and D3R knockout mice as well as wildtype controls
- E. Determine effects of APDs on kinetics of real-time glucose-stimulated insulin and dopamine release in wildtype and beta cell-specific D2R or D3R knockout mouse pancreatic islets

• **What was accomplished under these goals?**

**Generation of D3R-flox Mice by Homologous Recombination**



In the course of the reporting period for Year 1 of this award, we conducted studies to address each of the major goals of the project as follows:

**I. Metabolic characterization of hypothalamus-specific D2R and D3R knockout mice in the presence or absence of APD treatment**

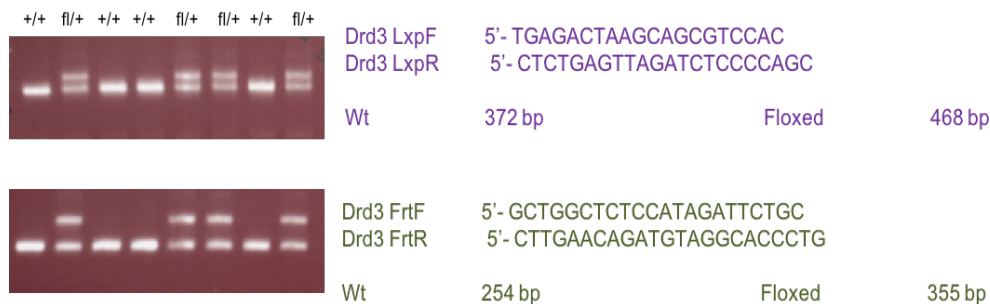
▪ In order to characterize the metabolic consequences of hypothalamus-specific knockout of D2R and D3R, we commenced construction of the transgenic D3R-flox mouse strain required for tissue-selective knockout of D3R. Consequently, in consultation with Dr. Claudia Schmauss (a consultant on this award) and Dr. Siu-Pok Yee of the University of Connecticut at Storrs, we examined the genomic sequences associated and flanking the *Drd3* gene corresponding to D3R. We then established a strategy to insert LoxP cassettes flanking the first exon of *Drd3*. In practice, the presence of these flanking cassettes permits Cre-recombinase mediated excision of this exon and therefore prevents transcription and translation of the entire D3R protein.

**Figure 1. Construction of targeted D3R-flox transgenic mouse strain.** We employed a recombineering approach to insert LoxP cassettes that flank the exon 1 coding region of the *Drd3* gene that encodes the D3R protein. Insertion of these cassettes has resulted in the generation of chimeric mice.

The first step in constructing this D3R-flox mouse strain was the design of a genomic targeting vector. The targeting vector was prepared by recombineering according to Lee et al. [Lee et al., (2001) *Genome Research* 13:476–484]. Briefly, we first retrieved approximately 12.2 kb of *Drd3* genomic sequence containing 8.3 kb of 5'-upstream, exon 1 and of 3.5 kb intron 1 sequence from the BAC, RP24-135K7, into the pDTA vector containing the PGK-DTA negative selectable marker by gap repair. We then inserted the 5' LoxP site approximately 4 kb upstream of the *Drd3* transcriptional start site in exon 1, followed by insertion of Frt-PGFneo-Frt-LoxP approximately 500 bp 3' downstream of exon 1. The final vector contains 5' and 3' arms of 4.2 and 3.2 kb, respectively. The vector was then linearized by NotI digestion, purified and electroporated into

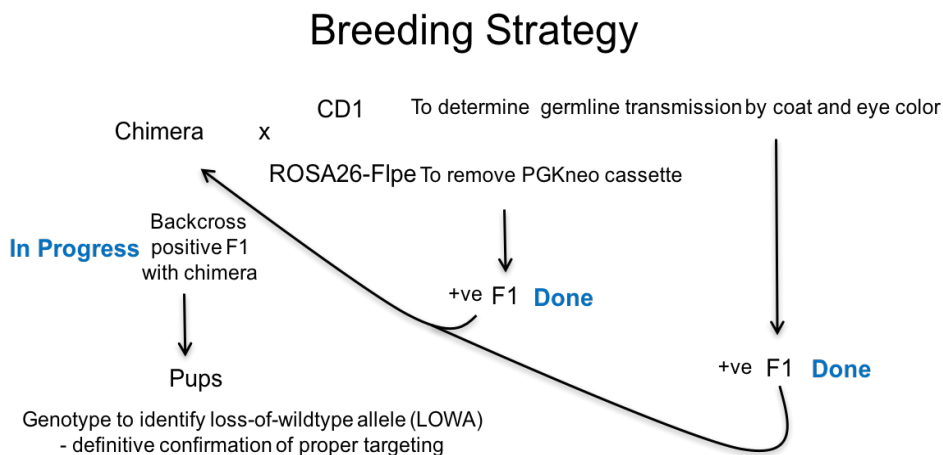
**Figure 2. PCR genotyping to confirm insertion of D3R LoxP sites.** We collected tail clippings from our newly generated D3R-flox chimeric mice and confirmed the presence of the LoxP sites flanking exon 1 of the *Drd3* gene using two independent PCR strategies (see primers in purple and green, respectively). The successful insertion of the LoxP elements was confirmed by the presence of two PCR bands versus a single band in the wildtype littermates.

**PCR Genotyping of D3R-flox Mice**



mouse ES cells derived from F1(129Sv/C57BL6j) blastocysts. Electroporated cells were cultured in the presence of the G418 antibiotic 48 hours post-electroporation. Drug-resistant colonies were picked and screened by long range PCR using primers corresponding to sequences outside the arms and specific to the 5' and 3' LoxP sites to identify targeted ES clones. Targeted ES clones were then expanded and further analyzed by long-range PCR for confirmation prior to using them for ES<->morula aggregation to generate chimeric animals (Figure 1). We further confirmed the presence of the inserted LoxP sites within the chimeric animals by additional PCR assays (Figure 2).

At present, the chimeric animals that we have generated are being bred with ROSA26-Flpe mice to remove the PGK neo cassette to generate the final conditional D3R-flox mouse strain (Figure 3).



**Figure 3. Breeding strategy to complete construction of the D3R-flox transgenic mouse strain.** Using our newly generated D3R-flox chimeric mice, we have conducted two separate crosses in parallel: (1) to CD1 mice to determine whether we have established successful germline transmission of the LoxP sites; and (2) to ROSA26Flpe mice to remove the PGKneo cassette. Presently, we are conducting the final backcrosses to complete the final targeted D3R-flox mice.

Upon generation of the strain, we will begin breeding them to Nkx2.1-cre mice which selectively express the Cre recombinase throughout the hypothalamus. The resulting Nkx2.1-cre hemizygous/D3R-flox homozygous mice will have selective knockout of D3R across the hypothalamus.

- In order to generate hypothalamus-specific D2R knockout mice, we bred our existing D2R-flox and Nkx2.1-cre hypothalamic expression driver mice to one another over the course of the year. This series of breeding steps required 12 months of breeding (concluding in September 2016) and permitted us to generate the final Nkx2.1-cre hemizygous/D2R-flox homozygous mice.
- Significantly, we have successfully secured IRB approval for all of our animal work during this reporting period.

## II. Metabolic characterization of pancreatic beta cell-specific D2R and D3R knockout mice in the presence or absence of APD treatment

- As of September 2016, we have completed construction of a transgenic mouse strain with transgenes for inducible D2R knockout specifically in pancreatic beta cells. In the course of designing the breeding strategy, we were able to use a new pancreatic beta cell-specific expression driver, Mip1-cre/ERT which has two important advantages over previous beta cell cre driver strains: (1) it is more specific to beta cells with virtually no off-target expression in other organ systems including brain; and (2) it is inducible, allowing us to avoid possible developmental effects that could confound our interpretation of the results. Following approximately 12 months of crosses, we have now successfully generated the final desired genotype of hemizygous cre; homozygous D2R-flox mice required to completely knockout expression of D2R selectively in pancreatic beta cells. With this transgenic mouse strain established, they can now be amplified in

numbers sufficiently powered to resolve potential effects of D2R absence on APDs' effects on pancreatic islet insulin and dopamine release.

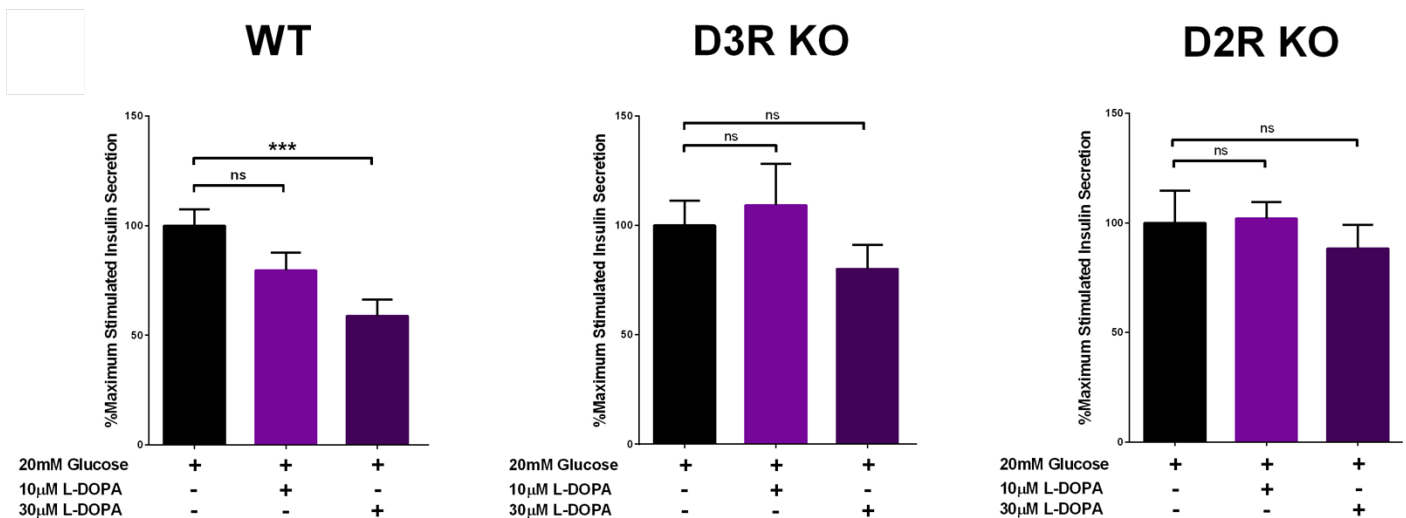
- The Mip1-cre/ERT mice are also being prepared for crosses to the new D3R-flox mouse strain in order to begin construction of an inducible beta cell-specific D3R knockout mouse line. We expect the crosses to begin in December 2016.

### III. Treatment with domperidone to determine whether peripheral D2R/D3R blockade alone can produce relevant metabolic disease

- We have been working closely with the Partnering PI of this project, Dr. Gary Schwartz, to optimize the dietary conditions responsible for inducing the development of insulin resistance (see Partnering PI report for further details). At present, preliminary data suggests that a diet combining high fat and high fructose in our C57Bl6/J genetic background is capable of inducing states of insulin resistance comparable to those seen in clinical populations, including in those treated with APDs. Once these dietary conditions are in place, we will henceforth use them as a baseline with which to compare effects of domperidone on development of metabolic disease both in wildtype as well as in beta cell-specific D2R or D3R knockout mice.

### IV. Determine the precise contributions of D2R and D3R to glucose-stimulated insulin and dopamine release using pancreatic islets from pancreatic beta cell-selective D2R and D3R knockout mice as well as wildtype controls

- To begin elucidating the precise role of pancreatic beta cell D2R in mediating glucose-stimulated insulin secretion (GSIS), we conducted insulin secretion assays using pancreatic islets from beta cell-specific D2R knockout mice and their wildtype littermate controls. As expected, we found that treatment of wildtype islets with the dopamine precursor, L-DOPA, had a concentration-dependent



**Figure 4. Absence of D2R or D3R expression in pancreatic islets attenuates dopaminergic inhibition of glucose-stimulated insulin secretion.** Glucose-stimulated insulin secretion from wildtype (WT) C57Bl/6J mouse pancreatic islets (WT, top left panel) is significantly decreased in response to treatment with 10  $\mu$ M L-DOPA (25% reduction,  $p=0.0341$ ) or 30  $\mu$ M L-DOPA (40% reduction,  $p=0.0012$ ) relative to stimulation with 20 mM glucose alone (90 min, 37°C). Under these same conditions, pancreatic islets from global D3R knockout (D3R KO, center panel) mice do not show concentration-dependent inhibition of GSIS when treated with 10  $\mu$ M L-DOPA ( $p>0.05$ ) or 30  $\mu$ M L-DOPA ( $p>0.05$ ) relative to stimulation with 20 mM glucose alone. Pancreatic islets from beta cell-selective D2R deletion (D2R KO, right panel) do not show concentration-dependent inhibition of GSIS when treated with 10  $\mu$ M L-DOPA ( $p>0.05$ ) or 30  $\mu$ M L-DOPA ( $p>0.05$ ) relative to stimulation with 20 mM glucose alone. All results represent  $n>3$  separate experiments and were conducted in triplicate.

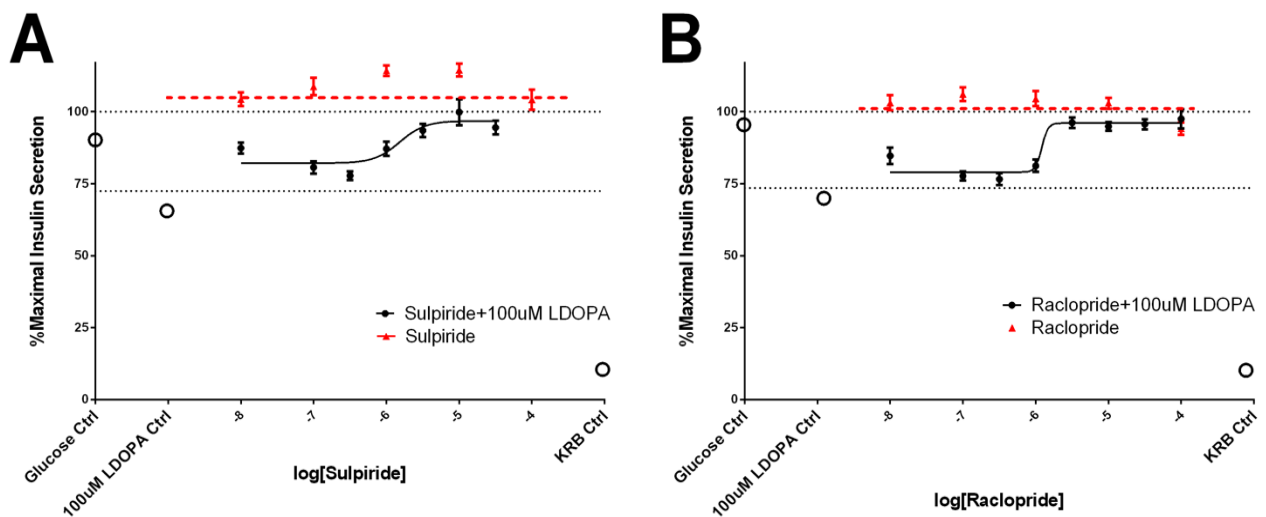
All results were normalized to effects of high glucose alone (20 mM) on GSIS and represent  $n>3$  separate experiments

increase in GSIS inhibition (Figure 4). By comparison, there was an attenuation of GSIS inhibition caused by L-DOPA treatment in the pancreatic islets from beta cell-selective D2R knockout mice

compared to the wildtype control islets ( $p > 0.05$ ). In parallel, we sought to assay the effects of D3R on pancreatic islet GSIS. However, since we do not yet have beta cell-selective D3R knockout mice, we used global D3R knockout mice which also lack D3R expression in the pancreas. Consequently, we found that absence of pancreatic D3R expression also significantly diminished L-DOPA-induced inhibition of GSIS in a concentration-dependent manner compared to the control ( $p > 0.05$ ). Our data therefore suggest that both D2R and D3R work in concert to mediate dopaminergic inhibition of GSIS. Dopamine secretion assays using pancreatic islets from D2R and D3R knockout mice are also currently underway and we hope to have complementary data in the coming months.

**V. Determine effects of APDs on kinetics of real-time glucose-stimulated insulin and dopamine release in wildtype and beta cell-specific D2R or D3R knockout mouse pancreatic islets**

- In preparation for examining the kinetics of glucose-stimulated insulin secretion in pancreatic islets, we have begun our studies by examining effects of APDs including sulpiride on GSIS in the pancreatic beta cell-derived INS-1E cell experimental system. We found that D2R/D3R blockade by sulpiride or a similar blocker, raclopride, alone had no significant effect on GSIS. Rather, these two drugs were most effective in blocking dopamine's inhibitory effects on GSIS following pre-incubation of the cells with L-DOPA, a precursor of dopamine (Figure 5). These data therefore suggest that beta cells require a priming step of the dopamine signaling machinery including at the receptor level in order to resolve effects of APD blockade of D2R and D3R.



**Figure 5. Effects of D2R/D3R by sulpiride and raclopride on glucose-stimulated insulin secretion (GSIS).** We examined the effects of D2R/D3R blockade by sulpiride or raclopride on GSIS in the insulin-secreting INS-1E cell line. **(A)** While increasing concentrations of sulpiride had no effect on their own (in red), following pre-incubation with the 100 μM dopamine precursor L-DOPA, increasing concentrations of sulpiride attenuated L-DOPA's inhibitory effects on GSIS (in black,  $EC_{50} = 1 \mu M$ ). **(B)** Like sulpiride, increasing raclopride concentrations had no effect on GSIS (in red), whereas increasing concentrations were able to block L-DOPA inhibition of GSIS. All results were normalized to effects of high glucose alone (20 mM) on GSIS and represent  $n > 3$  separate experiments.

• **What opportunities for training and professional development has the project provided?**

Nothing to Report.

• **How were the results disseminated to communities of interest?**

Work resulting from this award were presented at a national meeting the 54<sup>th</sup> Annual Meeting of the American College of Neuropsychopharmacology, (held in December 2015, Hollywood, FL). At this meeting, the data was presented both as an abstract as well as through a poster presentation. Moreover, data was presented at an international meeting, Dopamine 2016, (held in September 2016, Vienna, Austria). At



the Dopamine 2016 meeting, two abstracts based on these studies were published. This work was also disseminated to the scientific community via poster presentations. Presently, we are preparing three manuscripts based on our characterization of the mechanisms by which dopamine and dopamine D<sub>2</sub> and D<sub>3</sub> receptors mediate glucose-stimulated insulin secretion. We hope that the publication of these manuscripts will facilitate the dissemination of the experimental data derived from the aims of this project.

**I. What do you plan to do during the next reporting period to accomplish the goals?**

**I. Metabolic characterization of hypothalamus-specific D2R and D3R knockout mice in the presence or absence of APD treatment**

- In the next reporting period, we will conduct weekly measurement of weights and food consumption in hypothalamus-specific D2R (and wildtype littermate controls) treated with either with first-generation APD haloperidol or second-generation APD olanzapine (via i.p. administration). We will also measure serum fasting glucose and insulin levels in hypothalamus-specific D2R knockout mice and wildtype littermate control mice in the presence or absence of APD treatment; serum will be collected at weeks 13 and 26 of APD treatment.
- With the completion of construction of the D3R-flox mice, we will also begin crosses to establish hypothalamus-specific D3R knockout mice (Nkx2.1-cre hemizygous, D3R-flox homozygous mice). This process is expected to take approximately 10-12 months.

**II. Metabolic characterization of pancreatic beta cell-specific D2R and D3R knockout mice in the presence or absence of APD treatment**

- We will first characterize the quantity and duration of tamoxifen necessary to induce successful deletion of D2R in our inducible pancreatic beta cell-specific D2R knockout mice. Once we have confirmed deletion of D2R in pancreatic islets isolated from beta cells, we will begin characterizing the metabolic status of these animals from week 3 of life onwards following completion of weaning. Specifically, we will conduct weekly measurement of weights and food consumption in beta cell-specific D2R (and wildtype littermate controls) treated with either with first-generation APD haloperidol or second-generation APD olanzapine (via i.p. administration). We will also measure serum fasting glucose and insulin levels in hypothalamus-specific D2R knockout mice and wildtype littermate control mice in the presence or absence of APD treatment; serum will be collected at weeks 13 and 26 of APD treatment.
- In parallel with generation of beta cell-specific D2R knockout mice, we will also begin crosses to establish inducible pancreatic beta cell-specific D3R knockout mice (Mip1-cre/ERT hemizygous, D3R-flox homozygous mice). This process is expected to take approximately 10-12 months.

**III. Treatment with domperidone to determine whether peripheral D2R/D3R blockade alone can produce relevant metabolic disease**

- As reported above, once we and our Partnering PI have finalized the dietary conditions necessary to induce insulin resistance, we will use this diet to compare effects of domperidone on the rate of development of insulin resistance both in wildtype as well as in beta cell-specific D2R or D3R knockout mice. Besides insulin resistance, we will look at other markers of metabolic disease including adiposity, fatty liver and pancreatic beta cell mass.

**IV. Determine the precise contributions of D2R and D3R to glucose-stimulated insulin and dopamine release using pancreatic islets from pancreatic beta cell-selective D2R and D3R knockout mice as well as wildtype controls**

- Using pancreatic islets from inducible pancreatic beta cell-specific D3R knockout mice, we will validate our earlier findings demonstrating attenuation of L-DOPA inhibition of glucose-stimulated insulin secretion from global D3R knockout mice (see Figure 4). Moreover, we

will examine effects of other D2R/D3R agonists (including dopamine and quinpirole) on GSIS using beta cell-specific D3R knockout mice. These data would then be compared with those we have obtained from beta cell-specific D2R knockout mice and wildtype littermate controls.

- Using pancreatic islets from beta cell-specific D2R and D3R knockout mice, we will begin examining effects of these respective gene deletions on pancreatic islet dopamine secretion via an HPLC-based dopamine detection assay that we have developed.

**V. Determine effects of APDs on kinetics of real-time glucose-stimulated insulin and dopamine release in wildtype and beta cell-specific D2R or D3R knockout mouse pancreatic islets**

- We will validate our insulin secretion findings obtained from mouse pancreatic islets in human cadaveric pancreatic islets including assessment of effects of D2R agonists (dopamine, quinpirole) versus APD antagonists (haloperidol and olanzapine).
- We will treat pancreatic islets from wildtype and beta cell-specific D2R and D3R knockout mice with APDs (haloperidol, sulpiride, olanzapine and clozapine) to determine APD effects on glucose-stimulated insulin and dopamine release in a time-dependent manner. To do so, we will sample insulin and dopamine levels every 10 minutes which will permit determination of a kinetic curve for the respective release events.

#### **4. IMPACT**

• **What was the impact on the development of the principal discipline(s) of the project?**

The presentation of our preliminary results at scientific meetings including at a national conference, the 54<sup>th</sup> Annual Meeting of the American College of Neuropsychopharmacology, and at an international conference, Dopamine 2016, were instrumental in advancing the concept that APDs may act on peripheral dopaminergic targets. In presenting this work during talks and poster presentations, our findings were broadly disseminated to a broad scientific audience whose expertise spans multiple disciplines including neuroscience, endocrinology, cell biology and clinical medicine.

• **What was the impact on other disciplines?**

In the longer term, the knowledge resulting from our work may directly lead to development of better APDs free of metabolic side effects. This could significantly reduce serious morbidity and mortality from medication-associated type II diabetes and cardiovascular disease. Moreover, better understanding the mechanisms by which dopamine and dopamine receptors mediate insulin release may also significantly contribute to our fundamental understanding of obesity and lead to novel treatments. Since APD-induced metabolic disturbances also increase risks of developing type II diabetes and Alzheimer's disease, further elucidating the mechanisms of APD-induced weight gain may also lead to fundamental insights into the mechanisms for development of these disorders.

• **What was the impact on technology transfer?**

Nothing to Report.

• **What was the impact on society beyond science and technology?**

Nothing to Report.

#### **5. CHANGES/PROBLEMS**

Nothing to Report.

#### **6. PRODUCTS**

- **Publications, conference papers, and presentations**

- **Journal publications**

No peer-reviewed articles or papers appeared in scientific, technical or professional journals. However, presently three manuscripts are under preparation based on work resulting from this award. We expect to submit these manuscripts in the next 3-6 months.

- **Books or other non-periodical, one-time publications**

Nothing to report.

- **Other publications, conference papers, and presentations**

Data based on the studies originally proposed for this award were presented at a national meeting (54<sup>th</sup> Annual Meeting of the American College of Neuropsychopharmacology, December 2015, Hollywood, FL) as well as an international meeting (Dopamine 2016 meeting, September 2016, Vienna, Austria).

Abstracts based on the above poster presentations were published as follows:

1. Farino ZJ, Morgenstern TJ, Inbar BP, Maffei A, Harris PE, Donthamsetti P, Freyberg RJ, Kellendonk C, Schmauss C, Javitch JA, **Freyberg Z.** (2015) New Roles for Dopamine and Dopamine D<sub>2</sub>-like Receptors in Pancreatic Insulin Release. Abstract and poster presented at the 54<sup>th</sup> Annual Meeting of the American College of Neuropsychopharmacology, Hollywood, FL.
2. Farino ZJ, Morgenstern TJ, Inbar BP, Maffei A, Harris PE, Donthamsetti P, Freyberg RJ, Kellendonk C, Schwartz T, Schmauss C, Javitch JA, **Freyberg Z.** (2016) New perspectives on peripheral dopamine regulation of insulin release in the pancreas and its implications on antipsychotic drug-induced metabolic disease. Abstract, talk and poster presented at the Dopamine 2016 Conference, September 5th – 8th 2016 in Vienna, Austria.
3. Bonifazi A, Ellenberger M, **Freyberg Z,** Deschamps J, Janowsky A, Schwartz GJ, Pommier E, Rais R, Slusher B, Newman AH. (2016) Novel tools to investigate the role of dopamine D<sub>2</sub>/D<sub>3</sub> receptors in antipsychotic drug-induced metabolic disease. Abstract and talk presented at the Dopamine 2016 Conference, September 5th – 8th 2016 in Vienna, Austria.

- **Website(s) or other Internet site(s)**

Nothing to Report.

- **Technologies or techniques**

Nothing to Report.

- **Inventions, patent applications, and/or licenses**

Nothing to Report.

- **Other Products**

Nothing to Report.

## 7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

• Name:	Zachary Freyberg M.D., Ph.D.
• Project Role:	Principal Investigator
• Researcher Identifier (e.g. ORCID ID):	ORCID ID: 0000-0001-6460-0118
• Nearest person month worked:	3
• Contribution to Project:	Dr. Freyberg has designed and analyzed all experimental data in the areas of construction of the D3R-flox transgenic mouse strain and glucose-stimulated insulin secretion assays in beta cell-selective D2R knockout mice.
• Funding Support:	National Institutes of Health/NIDA K08 Mentored Career Development Award

• Name:	Zachary Farino
• Project Role:	Technician B
• Researcher Identifier (e.g. ORCID ID):	N/A
• Nearest person month worked:	3
• Contribution to Project:	Mr. Farino has performed cloning and molecular biological studies to construct optical reporters of insulin secretion and has conducted <i>in vitro</i> assays measuring insulin secretion from wildtype and dopamine D2R and D3R knockout mice.
• Funding Support:	N/A

• **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to Report.

• **What other organizations were involved as partners?**

Nothing to Report.

**8. SPECIAL REPORTING REQUIREMENTS**

• **Collaborative Awards**

We have worked closely with the Partnering PI of this award, Dr. Gary Schwartz. Dr. Schwartz has submitted a separate report independently of the Initiating PI that summarizes his progress over the course of the last reporting period (10/1/2015-09/30/2016).

**9. APPENDICES**

Below are the three abstracts presented as a poster at a national meeting (54<sup>th</sup> Annual Meeting of the American College of Neuropsychopharmacology, December 2015, Hollywood, FL) as well as talks at an international meeting (Dopamine 2016 meeting, September 2016, Vienna, Austria) (see the **Products** section).

- A. Abstract and poster presented at the 54<sup>th</sup> Annual Meeting of the American College of Neuropsychopharmacology, December 2015, Hollywood, FL:

## **New Roles for Dopamine and Dopamine D2-like Receptors in Pancreatic Insulin Release: Implications for Antipsychotic Drug Action Outside the Brain**

Zachary J. Farino, Travis J. Morgenstern, Benjamin P. Inbar, Antonella Maffei, Paul E. Harris, Prashant Donthamsetti, Robin J. Freyberg, Christoph Kellendonk, Claudia Schmauss, Jonathan A. Javitch, Zachary Freyberg

Departments of Psychiatry, Pharmacology & Medicine, Columbia University, New York, NY 10032  
Department of Psychology, Yeshiva University, New York, NY 10016

Sponsor: Jonathan A. Javitch

**Background:** Antipsychotic drugs (APDs) are some of the most widely used medications today. However, APDs also have prominent metabolic side effects, including substantial weight gain, glucose intolerance, and increase risks for type II diabetes and cardiovascular disease (Pramyothin and Khaothiar, 2010). Significantly, recent studies have demonstrated that all APDs cause metabolic disturbances (Fleischhacker *et al*, 2012). The single unifying property of all APDs is their blockade of dopamine D2-like receptors including D2 (D2R) and D3 receptors (D3R), suggesting their potential role in mediating these metabolic disturbances. Besides expression in brain regions implicated in metabolism, D2R and D3R are also expressed peripherally in insulin-secreting pancreatic beta cells (Rubi *et al*, 2005). Nevertheless, the precise roles of pancreatic DA and D2R/D3R remain poorly characterized. Thus, we have begun to investigate D2R/D3R-mediated effects on glucose-stimulated insulin secretion (GSIS) in mouse islets and INS-1E cells, a well-established rodent beta cell-derived cell line.

**Methods:** INS-1E cells and wildtype C57Bl6/J mouse-derived pancreatic islets were cultured in RPMI 1640 media (37°C, 5% CO<sub>2</sub>) prior to experimental use. GSIS was measured via a homogenous time-resolved fluorescence insulin assay we developed (Farino *et al.*, 2015). Cells or islets were first glucose-starved in Krebs-Ringer bicarbonate buffer (KRB; 1 h, 37°C) and then stimulated with 20 mM glucose in the presence or absence of additional drugs. Secreted insulin was measured from sample supernatant following drug incubation (30-90 min). To measure glucose-stimulated DA secretion, cells were pre-incubated with 30 uM L-DOPA (30 min, 37°C) followed by addition of additional drugs. Secreted DA in cell supernatant was quantified by high performance liquid chromatography (HPLC).

**Results:** In characterizing the DA biosynthetic machinery in beta cells, we found that human and rodent beta cells express the neuronal isoform of the vesicular monoamine transporter, VMAT2, which packages cytoplasmic DA into secretory vesicles. Consistent with this, we localized DA to insulin-containing dense core secretory vesicles by electron microscopy. Moreover, INS-1E cells took up L-DOPA and converted it to DA, leading to a 64% reduction in insulin secretion during GSIS in INS-1E cells with comparable effects in mouse islets. Treatment with exogenous DA also dose-dependently inhibited GSIS (IC<sub>50</sub>=1.28 uM) where 10 uM DA completely blocked GSIS. D2R/D3R played a key role in DA-mediated GSIS inhibition since D2R/D3R agonism by quinpirole mimicked DA's effects. Similarly, D2R/D3R blockade by raclopride or sulpiride attenuated L-DOPA's inhibitory effects on GSIS. We also examined D3R's individual contributions to these effects using D3R-specific blocker, R22, or via genetic D3R knockout. We found that inhibition of D3R function alone significantly attenuated effects of L-DOPA and DA on GSIS inhibition. Lastly, we showed that D2R/D3R agonism by quinpirole also significantly enhanced glucose-stimulated DA release, suggesting a feed-forward mechanism to potentiate DA's inhibitory effects on GSIS.

**Conclusions:** These results reveal that peripheral pancreatic DA and D2R/D3R receptors play important roles in metabolism through their inhibitory effects on GSIS. Consequently, by blocking peripheral pancreatic D2R/D3R, APDs override the DA-dependent negative feedback mechanism that mediates GSIS. This opens the possibility that besides their action in the central nervous system, APDs' actions on peripheral D2R/D3R may play a key role in causing the metabolic disturbances observed clinically.

**Disclosure:** Nothing to disclose.

B. Abstract, talk and poster presented at the Dopamine 2016 meeting, September 2016, Vienna, Austria:

**New perspectives on peripheral dopamine regulation of insulin release in the pancreas and its implications on antipsychotic drug-induced metabolic disease**

Zachary J. Farino, Travis J. Morgenstern, Benjamin P. Inbar, Antonella Maffei, Paul E. Harris, Prashant Donthamsetti, Robin J. Freyberg, Christoph Kellendonk, Claudia Schmauss, Jonathan A. Javitch, Zachary Freyberg

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Antipsychotic drugs (APDs) are some of the most widely used medications today. However, APDs also have prominent metabolic side effects, including substantial weight gain, glucose intolerance, and increase risks for type II diabetes and cardiovascular disease. Recent studies have shown that all APDs cause metabolic disturbances. The single unifying property of all APDs is their blockade of dopamine (DA) D2-like receptors including D2 (D2R) and D3 receptors (D3R), suggesting their potential role in mediating these metabolic disturbances. Besides expression in brain regions implicated in metabolism, D2R and D3R are also expressed peripherally in insulin-secreting pancreatic beta cells. Nevertheless, the precise roles of pancreatic DA and D2R/D3R remain poorly characterized. Thus, we have begun to investigate D2R/D3R-mediated effects on glucose-stimulated insulin secretion (GSIS) in mouse islets and INS-1E cells, a well-established rodent beta cell-derived cell line. In characterizing the beta cell DA biosynthetic machinery, we found that human and rodent beta cells express the neuronal isoform of the vesicular monoamine transporter, VMAT2, which packages cytoplasmic DA into secretory vesicles. Moreover, INS-1E cells took up L-DOPA and converted it to DA, leading to a 64% reduction in insulin secretion during GSIS, with comparable effects in mouse islets. Exogenous DA also inhibited GSIS ( $IC_{50}=1.28 \mu M$ ) where 10  $\mu M$  DA completely blocked GSIS. D2R/D3R played a key role in DA-mediated GSIS inhibition since D2R/D3R agonism by quinpirole mimicked DA's effects. Similarly, D2R/D3R blockade by raclopride or sulpiride attenuated L-DOPA's inhibitory effects on GSIS. We also examined individual contributions of D2R and D3R to these effects using D3R-specific blocker, R22, or via genetic D2R or D3R knockout (KO). Inhibition of D3R function alone significantly attenuated effects of L-DOPA and DA on GSIS inhibition which was confirmed by KO; beta cell-selective D2R KO islets showed similar effects. Lastly, we showed that D2R/D3R may mediate these effects through direct actions on beta cell DA secretion. These results reveal that peripheral pancreatic DA and D2R/D3R receptors play important roles in metabolism. This opens the possibility that besides their action in the CNS, APD actions on peripheral D2R/D3R may play a key role in causing the metabolic disturbances observed clinically.

C. Abstract, talk and poster presented at the Dopamine 2016 meeting, September 2016, Vienna, Austria:

Novel tools to investigate the role of dopamine D2/D3 receptors in antipsychotic drug-induced metabolic disease

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Antipsychotic drugs (APDs) are the most widely prescribed medications for treatment of major psychiatric illnesses. Despite their clinical utility, APDs cause significant metabolic side effects and increased risks for type II diabetes with consequent high rates of treatment discontinuation. The ubiquitous trait amongst effective clinical APDs is their antagonism at dopamine D2R and D3R playing a role in the mediation of APD-induced metabolic side effects. Evidence suggests that dopamine (DA) signaling through D2R and D3R in the central nervous system (CNS) mediates appetite and feeding behavior. Because both D2R and D3R are also expressed peripherally in both human and rodent insulin secreting pancreatic beta cells, DA signaling outside the CNS may provide an additional mechanism for systemic metabolic regulation and development of APD-induced metabolic disease. Activation of these receptors in beta cells mediates a negative feedback where DA co-released with insulin inhibits further insulin secretion. Chronic blockade of pancreatic D2R or D3R by APDs may result in hyperinsulinemia and diminished insulin sensitivity. Stimulation of beta cell D2R and/or D3R may mitigate or reverse some of these APD-induced effects. To avoid exacerbating psychosis in clinical populations by countering APDs' actions in the CNS, we have sought to design peripherally-limited D2R or D3R agonists that do not penetrate the blood-brain barrier via quaternization at the basic nitrogen. In binding studies using [<sup>3</sup>H]7-OHDPAT in HEK293 cells stably transfected with hD2R and hD3R most of the quaternary ammonium salts showed only a 4 to 10-fold loss of affinity at the D2R retaining agonist profiles in mitogenesis studies when compared with their parent molecules. The quaternary salt of bromocriptine (D2R/D3R agonist recently approved by the FDA to improve glycemic control in type II diabetes) retained very high affinity ( $K_i=2.9$  nM) for D2R when compared with bromocriptine itself ( $K_i=0.7$  nM). We examined these quaternary salts and their parent drugs in beta cell islet assays to determine their effects on glucose-stimulated insulin release. Bromocriptine and its methiodide analogue were also evaluated for mouse microsomal metabolism as well as for i.v. pharmacokinetic and blood/brain plasma ratios. Bromocriptine methiodide is currently being evaluated in *in vivo* metabolic analyses in mice including indirect calorimetry, food intake and body weight in the presence or absence of APD treatment.