

AWARD NUMBER: W81XWH-15-1-0556
PR141292

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

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CONTRACTING ORGANIZATION: University of Pittsburgh

REPORT DATE: DECEMBER 2020

TYPE OF REPORT: Final Technical Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

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1. REPORT DATE DECEMBER 2020			2. REPORT TYPE Final Report		3. DATES COVERED 09/30/2015 - 09/29/2020	
4. TITLE AND SUBTITLE Central and Peripheral Mechanisms of Antipsychotic Medication-Induced Metabolic Dysregulation					5a. CONTRACT NUMBER W81XWH-15-1-0556	
					5b. GRANT NUMBER PR141292	
					5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Zachary Freyberg M.D., Ph.D. E-Mail: freyberg@pitt.edu					5d. PROJECT NUMBER	
					5e. TASK NUMBER	
					5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Pittsburgh 3520 Fifth Avenue Pittsburgh, PA 15213-3320					8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012					10. SPONSOR/MONITOR'S ACRONYM(S)	
					11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited						
13. SUPPLEMENTARY NOTES						
14. ABSTRACT 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 ₂ (D2R) and D ₃ (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. We have completed construction of novel inducible transgenic hypothalamic- and pancreatic beta cell-specific D2R knockout (KO) mice and are finalizing construction of hypothalamic and pancreatic beta cell-selective D3R KO mice. Additionally, using pancreatic islets isolated from beta cell-specific 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. In parallel, we have developed novel assays for measurement of pancreatic alpha cell glucagon and shown that APDs act directly on alpha cells to significantly disturb glucagon release.						
15. SUBJECT TERMS						
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON	
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC	
Unclassified	Unclassified	Unclassified	Unclassified	9	19b. TELEPHONE NUMBER (include area code)	

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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 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 D₂ (D2R) and D₃ (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 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 has elucidated 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 D₂ Receptor (D2R)
4. Dopamine D₃ Receptor (D3R)
5. Insulin
6. Glucagon
7. Glucose-stimulated insulin secretion (GSIS)
8. Diabetes
9. 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

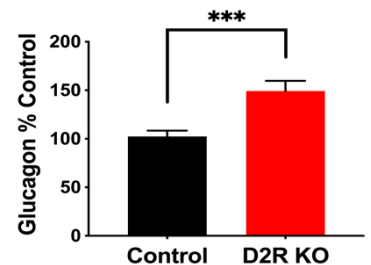
- C. Treatment with domperidone to determine whether peripheral D2R/D3R blockade alone can produce relevant metabolic disease
- D. Determine the precise contributions of D2R and D3R to glucose-stimulated insulin and dopamine release using pancreatic islets from pancreatic beta cell-selective D2R and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

In the course of the reporting period for the final year 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

- To characterize the metabolic consequences of hypothalamus-specific knockout (KO) of D2R and/or D3R, as a first step, we successfully constructed a D3R-flox mouse strain to knock out D3R expression selectively in the hypothalamus. We have now completed back-crossing of the D3R-flox mice to C57BL/6J mice for 10 generations in order to make the D3R-flox strain congenic to the C57BL/6J genetic background, a widely accepted background widely used in metabolic studies. With these mice in hand, we commenced creating hypothalamus-selective and pancreatic beta cell-selective D3R KO mice.
- Partnering PI, Dr. Gary Schwartz is now actively characterizing the potential metabolic consequences of the resulting loss of hypothalamic D2R expression within the hypothalamus-specific D3R KO mouse strain. Furthermore, together with Dr. Schwartz, we are determining effects of this hypothalamus-specific D2R KO on APD-induced metabolic disturbances by treating the hypothalamic KO mice and their wildtype littermate controls with either olanzapine, haloperidol or vehicle control.
- We have successfully renewed IACUC 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

- We discovered that islets from D2R knockout mice (KO) exhibited significantly elevated glucagon secretion in response to glucose stimulation as indicated by a 50% increase compared to islets from wildtype littermates ($p=0.0009$) (**Figure 1**). These results suggest that the derangements in insulin secretion that we observed in the beta cell-specific D2R KO islets also have a paracrine effect on islet glucagon secretion. These results may explain the chronic hyperglucagonemia also observed in both diabetes as well as in response to APD therapy. Such hyperglucagonemia may be an important contributor to the hyperglycemia described clinically.
- The Mip1-Cre/ERT mice used to drive beta cell-specific expression of the Cre recombinase are now being prepared for crosses to the new D3R-flox mouse strain as the D3R-flox strain is fully isogenic with C57BL/6J (see above). The aim will be to begin construction of an inducible beta cell-specific D3R knockout mouse line.

Figure 1. Beta cell-specific D2R knockout increases paracrine glucagon secretion. We examined effects of beta cell-specific D2R knockout (KO) glucose-stimulated glucagon secretion (GSIS) in pancreatic islets from D2R KO ($n=3$) vs wildtype littermate control mice ($n=3$). Glucagon release was increased by 50% in KO islets compared to islets from littermate controls ($p=0.0009$). All data conducted in triplicate and normalized to % glucagon secretion relative to control. Results are represented as mean \pm SEM.

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

- Cyclic AMP (cAMP) signaling is integral to regulation of glucose-stimulated insulin secretion (GSIS) where intracellular cAMP levels increase in response to glucose stimulation as a key signaling step to facilitate hormone release. Consequently, we investigated the effects of peripheral D2R/D3R blockade by domperidone on intracellular cAMP biosynthesis during GSIS. We found that increasing domperidone concentrations had no significant effects on cAMP levels in the beta cell-derived INS-1E cells (**Figure 2A**). In contrast, the same domperidone concentrations raised overall insulin secretion at nanomolar concentrations (**Figure 2B**), consistent with a role for D2R/D3R signaling in modulation of GSIS.

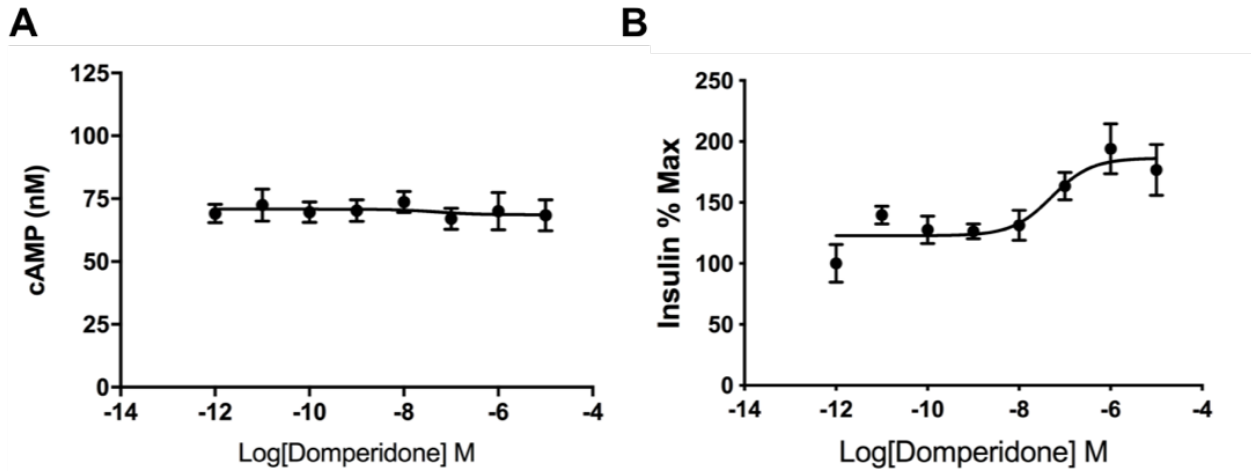


Figure 2. Domperidone raises glucose-stimulated insulin secretion independently of cyclic AMP (cAMP) levels. (A) Domperidone treatment of rat beta-cell derived INS-1E cells did not produce significant changes in intracellular cAMP levels ($p > 0.05$). (B) Domperidone treatment of pancreatic islets isolated from wildtype mice significantly raised glucose-stimulated insulin secretion, $EC_{50} = 51.64$ nM in spite of the absence of drug-induced changes in cAMP. Insulin values were normalized to % maximal insulin secretion. All data conducted in triplicate. Results are represented as mean \pm SEM.

IV. Determine the precise contributions of D2R and D3R to glucose-stimulated insulin and dopamine release

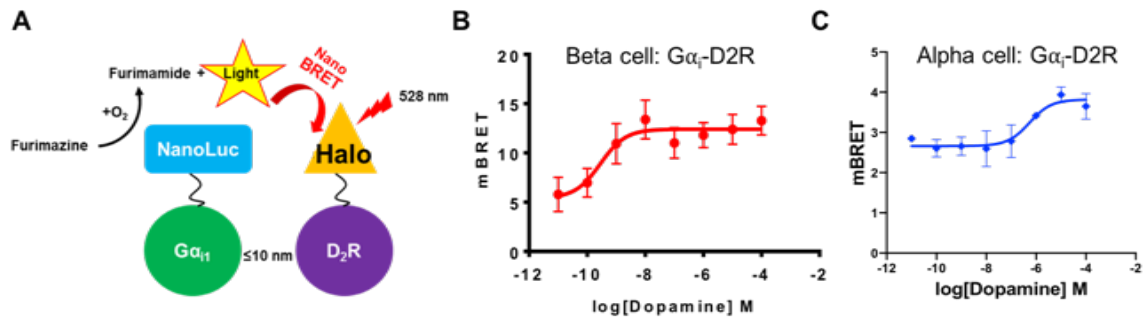


Figure 3. DA stimulation triggers G protein recruitment to D2R by nanoBRET in beta- and alpha-cells. (A) Schematic of nanoBRET: D2R is tagged with a dye via a HaloTag. Luminescent nanoLuciferase excites the HaloTag dye when the molecules are in proximity, producing fluorescence. (B) DA stimulation in INS-1E cells expressing D2R-HaloTag and NanoLuc- $G\alpha_{i1}$ shows increasing $G\alpha_{i1}$ recruitment to D2R ($EC_{50} = 0.28$ nM). (C) DA stimulation also recruits $G\alpha_{i1}$ to D2R in α TC1-6 cells ($EC_{50} = 500$ nM). Mean \pm SEM.

- The abilities of D2R and D3R to recruit G protein $G\alpha_i$ and Arr2 are critical for initiating the intracellular signaling important for modulating hormone secretion. Significantly, APDs can

disrupt these recruitments, providing a testable new molecular mechanism for these drugs' metabolic side effects in alpha- and beta-cells. Thus, to study recruitment of $G\alpha_i$ and Arr2 to D2R or D3R in α - and β -cells, we employed the recently developed nanoBRET (nano Bioluminescence Resonance Energy Transfer) approach (**Figure 3A**). The major advantage of nanoBRET is its significantly improved sensitivity, allowing us to detect transient and/or weak intermolecular interactions. Moreover, while virtually all studies of DA signaling have been conducted in non-islet cell types (*e.g.*, HEK cells), we will study signaling directly in islet cell types. As proof-of-principle, we used nanoBRET to show $G\alpha_i$ recruitment to D2R during DA stimulation in the INS-1E β -cell line (**Figure 3B**) and the mouse α TC1-6 alpha-cell line (**Figure 3C**), opening the door to detect APD-induced changes.

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

Nothing to Report.

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

Our results were disseminated to communities of interest at national and international scientific meetings (see below). Collectively, presenting recent findings stemming from this project were instrumental in advancing the concept that APDs may act on peripheral dopaminergic targets. In presenting this work during talks, abstracts 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. Furthermore, we have also published our results extensively. 7 publications detailing our results from this project were published in high-impact journals including *Science Advances* with two additional papers under revision. In addition to the work already published, we presently have five manuscripts under preparation directly based on work resulting from this award. We expect to submit these manuscripts in the next 6-12 months.

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

This no longer applicable as this is the final report for this award.

4. **IMPACT**

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

We presented our preliminary results at scientific meetings including at national and international conferences: 2020 Islet Biology Keystone meeting (2020), Dopamine 2020 meeting (2020), Virtual Dopamine meeting (2020), Annual Sleep & Circadian Science Research Meeting (2019), and 58th annual meeting of the American College of Neuropsychopharmacology meeting (2019). These presentations were instrumental in advancing the concept that APDs may act on peripheral dopaminergic targets which is a topic directly relevant to endocrinology and psychiatry – the two principal disciplines associated with this project. This has begun generating considerable interest within these two fields.

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

In presenting results from this project, our findings were also broadly disseminated to a broad scientific audience beyond endocrinology and psychiatry. The results were communicated to diverse audiences whose expertise spanned multiple disciplines including neuroscience, cell biology and clinical medicine. By appealing to a broader audience, this may foster in the longer-term new knowledge that leads to development of better APDs free of metabolic side effects. Ultimately, such a development could significantly reduce serious morbidity and mortality from medication-associated type II diabetes and cardiovascular disease. Moreover, better understanding the mechanisms by which DA and DA 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

There have been no changes in the scope of work since the last reporting periods and therefore the SOW remains the same as originally defined.

6. PRODUCTS

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

Journal publications

Data based on the work resulting from this award has appeared in the following publications:

1. Wei H, Zapata R, Lopez-Valencia M, Farino ZJ, Aslanoglou D, Benner V, Osborn O, **Freyberg Z (co-corresponding author)**, McCarthy MJ. Dopamine D₂ receptor signaling modulates pancreatic beta cell circadian rhythms. *Psychoneuroendocrinology* 2020. 113, 104551. doi:10.1016/j.psyneuen.2019.104551. PubMed PMID: 31884319.
2. Carter SD, Hampton CM, Langlois R, Melero R, Farino ZJ, Li W, Calderon MJ, Wallace CT, Tran NH, Grassucci RA, Siegmund SE, Pemberton J, Morgenstern TJ, Aguilar JI, Greenberg NL, Levy ES, Yi E, Mitchell WG, Rice WJ, Wigge C, Pilli J, George EW, Aslanoglou D, Courel M, Freyberg RJ, Javitch JA, Area-Gomez E, Shiva S, Bartolini F, Volchuk A, Murray SA, Aridor M, Fish KN, Walter P, Balla T, Fass D, Wolf SG, Watkins SC, Carazo JM, Jensen GJ, Frank J, **Freyberg Z**. Ribosome-Associated Vesicles: a dynamic vesicular endoplasmic reticulum in secretory cells. *Science Advances* 2020. 6(14), eaay9572. doi: 10.1126/sciadv.aay9572. PubMed PMID: 32270040. PMCID: PMC7112762.
 - a. Commentary in: Farrell RJ, Ryan TA. Local sourcing of secretory proteins in faraway places. *Trends Neurosci.* 2020. 43(9):649-650. doi: 10.1016/j.tins.2020.06.004. Epub 2020 Jun13. PubMed PMID: 32546404.
3. Isaacson R, Beier J, Khoo N, Freeman B, **Freyberg Z**, Arteel G. Olanzapine-induced liver injury in mice: aggravation by high-fat diet and protection with sulforaphane. *J Nutritional Biochemistry* 2020. Apr 8;81:108399. doi: 10.1016/j.jnutbio.2020.108399. PMID: 32388251.
4. Fiala T, Wang J, Dunn M, Šebej P, Choi SJ, Nwadiibia EC, Fialova E, Martinez DM, Cheetham CE, Fogle KJ, Palladino MJ, **Freyberg Z**, Sulzer D, Sames D. Chemical targeting of voltage sensitive dyes to specific cells and molecules in the brain. *Journal of the American Chemical Society* 2020. May 12. doi: 10.1021/jacs.0c00861. PMID: 32395989.

5. **Freyberg Z**, Saavedra JM. Trace amines and trace amine-associated receptors: a new frontier in cell signaling. *Cell Mol Neurobiol.* 2020. 40(2) 189-190. doi:10.1007/s10571-020-00800-x. PubMed PMID: 32006222.
6. Buck SA, Torregrossa MM, Logan RW, **Freyberg Z**. Roles of dopamine and glutamate co-release in the nucleus accumbens in mediating the actions of drugs of abuse. *FEBS J.* 2020. Jul 23. doi: 10.1111/febs.15496. PubMed PMID: 32702182.
7. Gayden J, **Freyberg Z**. Commentary: Ghrelin promotes midbrain neural stem cells differentiation to dopaminergic neurons through the Wnt/ β -catenin pathway. *Frontiers in Cellular Neuroscience – Cellular Neuropathology.* 2020. DOI: 10.3389/fncel.2020.00248.
8. Aslanoglou D, Bertera S, Sanchez-Soto M, Free RB, Lee J, Zong W, Xue X, Shrestha S, Brissova M, Logan RW, Wollheim C, Trucco M, Yechoor V, Sibley D, Bottino R, **Freyberg Z**. Dopamine regulates pancreatic glucagon and insulin secretion via adrenergic and dopaminergic receptors. *Translational Psychiatry Under revision*
9. Zhu Y, Sun D, Schertel A, Ning J, Fu X, Guo P, Watson AM, **Freyberg Z**, Zhang P. Serial cryoFIB/SEM reveals profound cytoarchitectural disruptions caused by a pathogenic mutation in Leigh syndrome patient cells. *Structure Under revision*

In addition to the work already published or under revision, we presently have five manuscripts in preparation and expect to submit these manuscripts in the next 6-12 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 the following meetings and presentations:

1. Aslanoglou D, Bertera S, Sanchez-Soto M, Lee J, Yechoor V, Brissova M, Free RB, Sibley D, Bottino R, **Freyberg Z**. (2020) Dopamine and norepinephrine regulate α -cell glucagon secretion. Abstract and poster presented at the 2020 Islet Biology: From Gene to Cell to Micro-Organ Keystone Symposium, Santa Fe, NM.
2. Buck SA, De Miranda BR, Greenamyre JT, Fish KN, Glausier JT, Lewis DA, **Freyberg Z**. (2020) Differential co-expression of tyrosine hydroxylase and vesicular glutamate transporter 2 in human and rodent aging and a rotenone model of Parkinson's disease. Abstract and poster presented at Dopamine 2020 Meeting, Montreal, Canada.
3. Buck SA, Villeneuve M, Bhatte SH, Childers VC, Rubin SA, Cheetham CEJ, **Freyberg Z**. (2020) Age-related dopamine neuron degeneration and the protective role of vesicular glutamate transporter expression in *Drosophila*. Abstract and poster presented at the Virtual Dopamine Meeting.
4. Aslanoglou D, Bertera S, Sanchez-Soto M, Free RB, Lee J, Zong W, Xue X, Shrestha S, Brissova M, Logan RW, Yechoor V, Sibley DR, Bottino R, **Freyberg Z**. (2020) New peripheral dopaminergic mechanisms of antipsychotic drug-induced metabolic disturbances. Abstract and talk presented at the 59th Annual Meeting of the American College of Neuropsychopharmacology, Virtual.

Additionally, Dr. Freyberg was an invited speaker at the following seminars where he presented the work produced from this funded work:

1. Invited speaker, Presynaptic Mechanisms Nanosymposium, Neuroscience 2019, Washington D.C.; 2019
2. Invited speaker, 7th Annual Molecular Psychiatry Meeting, Molecular Psychiatry Association, San Francisco; 2019
3. Invited speaker, 5th Annual Sleep & Circadian Science Research Day, University of Pittsburgh, Pittsburgh, PA; 2019
4. Invited presenter, 58th annual meeting of the American College of Neuropsychopharmacology meeting, Orlando, FL; 2019
5. Invited presenter, Annual Pittsburgh Liver Research Center retreat, Pittsburgh, PA; 2020
6. Invited speaker, American Physical Society Annual Meeting, Denver, CO; 2020
7. Invited speaker, Dopamine 2020 Meeting, Montreal, Canada; 2020
8. Invited speaker, 2020 Gordon Research Conference on Membrane Transport Proteins: Biomedical Transporters: Physiology, Dysfunction and Targets of Pharmacotherapy, Barcelona, Spain; 2020
9. Invited speaker, 8th Annual Meeting of the Canadian Neurometabolic Club meeting, Montreal, Canada; 2020
10. Invited lecturer, Annual *Drosophila* Neurobiology Course, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 2020
11. Invited speaker, minipanel at 59th Annual Meeting of the American College of Neuropsychopharmacology, Virtual
12. Invited speaker, Organelle Cross Talk and Contact Sites Symposium, Cell Bio Virtual 2020 Annual Meeting of the American Society of Cell Biology, Virtual

• **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:	5
• 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:	Department of Defense Peer Reviewed Medical Research Program Investigator-Initiated Research (PR141292)

• Name:	Despoina Aslanoglou, Ph.D.
• Project Role:	Postdoctoral Researcher
• Researcher Identifier (e.g. ORCID ID):	N/A
• Nearest person month worked:	12
• Contribution to Project:	Dr. Aslanoglou has performed cloning and molecular biological studies, tissue culture as well as <i>in vitro</i> and <i>ex vivo</i> functional assays measuring insulin, glucagon and DA secretion from mouse and human pancreatic islets and hormone-secreting pancreatic cell-derived cell lines.
• Funding Support:	Department of Defense Peer Reviewed Medical Research Program Investigator-Initiated Research (PR141292)

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

The following grant

PR192466	DoD Discovery Award, “A New <i>In Situ</i> Cryo-Electron Microscopy Approach to Directly Visualize Mutations in Mitochondrial Disease”	PI, 15% effort The goal of this award is to directly visualize mitochondrial disease in patient cells via <i>in situ</i> cryo-electron tomography	3/1/2020-2/28/2022	DoD \$312,847 (Direct: \$200,000)
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• **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.

9. APPENDICES

Below is the abstract presented at two international meetings including the 2020 Islet Biology: From Gene to Cell to Micro-Organ Keystone Symposium:

Title: Catecholamines regulate α - and β -cell secretion

Authors: Despoina Aslanoglou, Suzanne Bertera, Marta Sanchez Soto, R. Benjamin Free, Jeongkyung Lee, Vijay Yechoor, David Sibley, Rita Bottino, Ryan W. Logan, **Zachary Freyberg**

Glucagon and insulin are key hormonal regulators of glucose homeostasis. Moreover, disturbances in secretion of both hormones are characteristic features of diabetes mellitus (DM). However, the precise mechanisms controlling α -cell glucagon secretion and β -cell insulin release remain unknown. Growing evidence demonstrates that catecholamines like dopamine (DA) and norepinephrine (NE) are critical metabolic modulators. Indeed, we previously showed that β -cells readily produce and secrete DA in response to uptake of the DA precursor L-DOPA. The resulting DA acts as a negative regulator of insulin secretion. We therefore asked whether catecholamines also regulate glucagon secretion, since α -cells also express dopamine receptors and the catecholaminergic biosynthetic machinery.