VERTICES

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Letter from the Editor

Dear Reader,

Welcome to the fifth issue of Vertices: Duke's Undergraduate Research Journal. It has been a privilege watching our peer review team grow throughout my time at Duke. Four years ago, Vertices was an article repository with no formal publishing structure. Now, we are publishing our 5th issue, alongside a team of 30 peer reviewers, senior editors, Georgetown peer reviewers, and faculty reviewers. I am incredibly proud of our team and the collective growth we have made.

In this current issue, we are excited to showcase interdisciplinary research on aquatic animal models, female reproduction, and ChatGPT's impact on cognitive flexibility. Our first article explores how aspects of female reproduction—specifically the progesterone receptor—are regulated through the human genome. Our second piece is a two-part article using aquatic animals to explore foundational biological processes—including exercise, feeding, muscle control, and metabolic rate. Our final article investigates the relationship between ChatGPT usage and cognitive flexibility in college students.

My involvement with Vertices these last four years has been an outstanding part of my Duke experience. I can't wait to watch this organization and our new team continue to develop and progress. Please join in my excitement as you read this thought-provoking publication from our 2024 Vertices team!

Sincerely,

Julia Davis, Editor in Chief

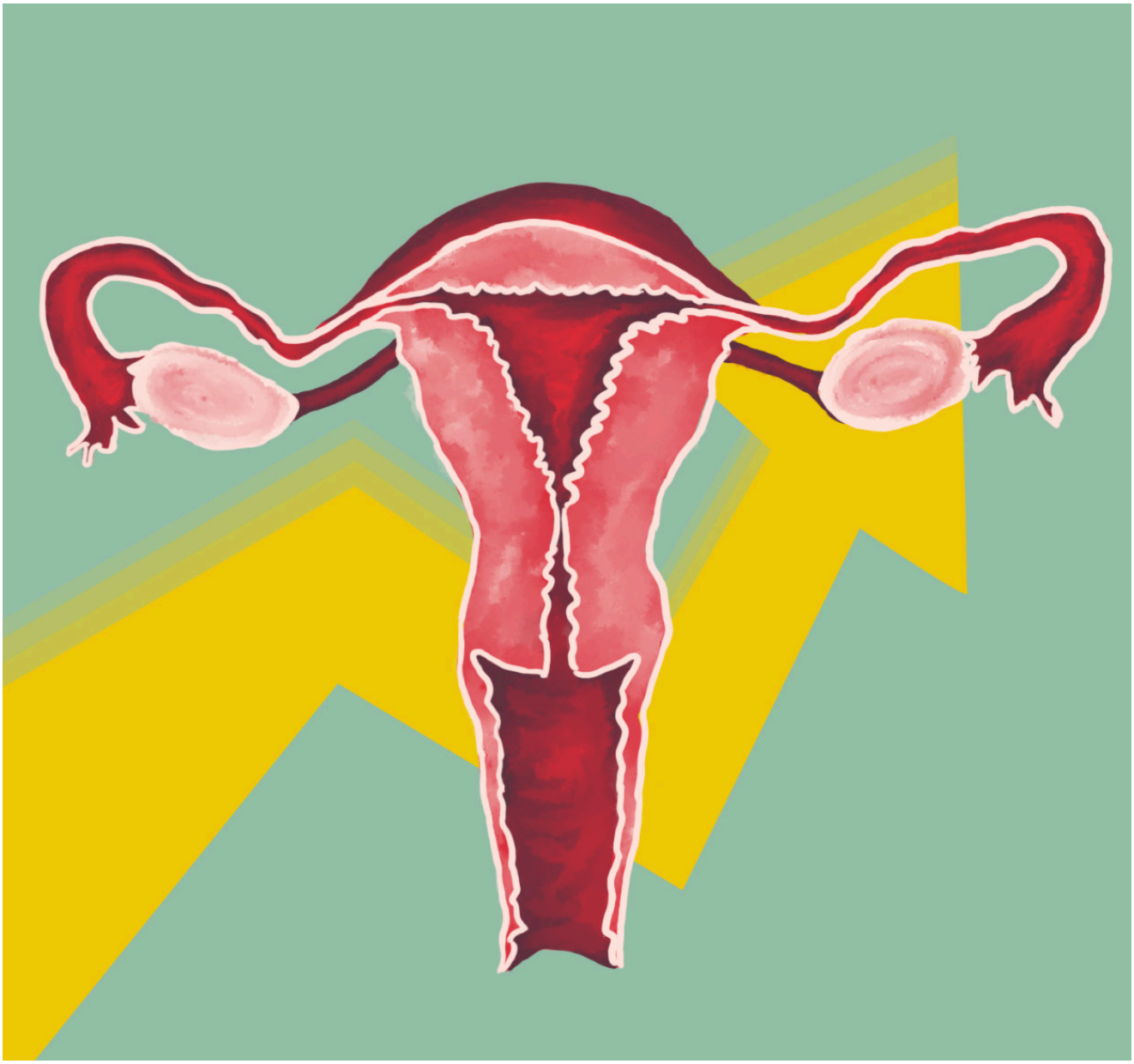
Identification of cis-acting elements that regulate expression of the progesterone receptor gene PGR in the uterus.

Skylar Montague Redecke

Volume 3, Issue 1

Article Synopsis

The hormone progesterone, alongside its receptor PGR, is a master regulator for female reproductive function, orchestrating processes like pregnancy establishment, maintenance, and labor. When PGR levels are misregulated, progesterone resistance can occur, which is an underlying factor in many reproductive pathologies including endometriosis and infertility. Despite the importance of PGR in the reproductive tract, very little is known about how it is regulated. In this study, elements of the genome that are critical for regulating PGR are identified to gain insight into how it becomes disrupted, and how we can restore its delicate balance.



Graphic by Gaby Dunn

Identification of cis-acting elements that regulate expression of the progesterone receptor gene PGR in the uterus.

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Abstract

The steroid hormone progesterone regulates a wide range of functions in the uterus, including menstruation, pregnancy establishment, pregnancy maintenance, and labor. The cellular effects of progesterone are mediated by the nuclear progesterone receptor (PGR). Progesterone resistance, a decreased cellular response to progesterone, is implicated in a wide range of pathologies including endometriosis, preterm birth, and endometrial cancer. Up to 10% of females within reproductive age are impacted by endometriosis, and no cure is available. This study hypothesizes that misregulation of PGR by upstream regulators is a potential cause of progesterone resistance. However, much remains unknown about the regulation of PGR expression, including the location of enhancers and the identity of transcriptional regulators that control PGR expression. In this study, candidate regulatory elements of PGR were identified by integrative analysis of genomic data, and their function was tested using the CRISPR activation system, with PGR mRNA levels as the readout. Using these methods, two PGR enhancers located at the PGR 3' untranslated region and 200 kbs upstream of the PGR transcription start site were identified. These enhancers permitted the identification of candidate upstream regulators of PGR expression in the uterus, including GATA2, ARID1A, KLF9, and ESR1. Activation of ESR1 increased PGR mRNA levels in the presence of estradiol in cultured uterine cells. Through the discovery of PGR regulators, we can improve our understanding of molecular mechanisms of progesterone resistance. Ultimately, these regulators could be evaluated in cases of progesterone resistance and targeted in the development of therapies.

INTRODUCTION

Progesterone is a steroid hormone synthesized by the ovaries, corpus luteum, and placenta (J. D. Graham & C. L. Clarke, 1997) and plays a central role in the regulation of biological processes throughout uterine tissues: the two major layers of the uterus, the endometrium and the myometrium. The myometrium is the smooth muscle component of the uterus that protects the developing fetus throughout gestation and generates contractile forces at the time of labor to deliver the fetus (Carsten, 1968). The endometrium forms the inner lining of the uterus, composed of the outer epithelium and inner stromal compartment. The epithelium thickens in anticipation of pregnancy and is the site of implantation if pregnancy is established. In the absence of pregnancy, menstruation occurs, resulting in the shedding of the endometrial lining (Critchley et al., 2020). If pregnancy is established, progesterone signaling triggers the differentiation of the stromal compartment into decidual cells (a process known as decidualization) that support embryo growth and maintain early pregnancy (Ng et al., 2020). Progesterone signaling is central to the two major layers of the uterus, the endometrium and the myometrium, regulating a host of biological processes including menstruation, ovulation, embryo implantation, uterine growth, and labor (J. D. Graham & C. L. Clarke, 1997).

Progesterone resistance, a decreased cellular response to progesterone throughout the uterus, is implicated in a wide range of pathologies including infertility, endometriosis, endometrial cancer, and adenomyosis (MacLean & Hayashi, 2022). Endometriosis occurs when endometrial tissue invades neighboring compartments, such as the fallopian tubes, ovaries, or vagina, causing chronic pelvic pain, infertility (Zondervan et al., 2018), nausea, fatigue, and bloating (WHO, 2023). The World Health Organization estimates that up to 10% of girls and women within reproductive age, or 190 million females worldwide, are impacted by endometriosis (WHO, 2023). To temporarily relieve pain, administration of hormone treatment to inhibit ovulation and menstruation, or surgical removal of lesions can be employed, but there is currently no cure (Vercellini et al., 2014). Furthermore, endometriosis can lead to subfertility or infertility, with the risk of infertility increasing two-fold in patients under the age of 35 with endometriosis compared to unaffected peers (Prescott et al., 2016). When endometrial tissues grow into the myometrial layer of the uterus, adenomyosis occurs which leads to heavy menstrual bleeding, increased risk of anemia, dyspareunia (pain during sex), decreased fertility, severe period cramps, and an enlarged uterus (Mildred R Chernofsky, 2016).

The cellular effects of progesterone are mediated by the nuclear progesterone receptor (PGR) which exists as two isoforms (PGRA and PGRB) encoded by the same gene via alternate promotors (Kastner et al., 1990; O'Malley & Schrader, 1972). Upon binding of progesterone to PGR, the dimerized receptor-ligand complex translocates to the nucleus and binds to the genome as a transcription factor (TF) to regulate a wide range of gene expressions (J. Graham & C. Clarke, 1997; J. D. Graham & C. L. Clarke, 1997; O'Malley & Schrader, 1972). The PGRA and B isoforms share the majority of their protein product structure. The conserved domains across the two include an N-terminal domain, a DNA binding domain, a ligand binding domain, and two transactivating domains (AF1 and AF2) that form the interface that interacts with coregulators (Mulac-Jericevic & Conneely, 2004). PGRB has an additional 164 amino acids at the N-terminal domain which codes for the transactivation domain, AF3, which is thought to explain the unique gene regulatory actions of each isoform throughout the uterus (Sartorius et al., 1994; Tora et al., 1988). In many cellular contexts, PGR isoforms coexist and interact via homo- or heterodimers, with the stoichiometric ratio of each isoform likely determining the genetic and molecular response to progesterone hormone signaling (DeMayo & Lydon, 2020).

PGR regulates a host of programs in the female reproductive tract required for embryo implantation, stromal decidualization, myometrial remodeling, and parturition. At ovulation, the newly formed corpus luteum secretes progesterone which antagonizes estrogen-induced proliferation of the endometrium (DeMayo & Lydon, 2020). Through the Pgr-Ihh-Nr2f2-Hand2 axis, the outer lining of the endometrial compartment, the epithelium, transitions to a receptive state, allowing for embryo implantation and subsequent invasion into the uterus (Oh et al., 2023). During this time, crosstalk between the stromal and epithelial compartments of the endometrium through the Pgr-Ihh-Nr2f2 axis regulates the differentiation of endometrial stromal cells into decidual cells, a process known as decidualization (Lee et al., 2010; Takamoto et al., 2002). Decidual cells create an immunotolerant environment and nourish the embryo until the placenta forms (Gellersen & Brosens, 2014). Throughout pregnancy, the myometrium undergoes drastic development, increasing in size up to 5-fold to accommodate the growing fetus (Carsten, 1968), while developing into a synchronous contractile unit capable of generating rhythmic and intense contractions (Csapo, 1981). Throughout gestation, high levels of progesterone ligand bind to PGRB, the dominant isoform at this time, to repress contractile genes and maintain a non-contractile myometrial state as the fetus develops (DeMayo & Lydon, 2020; Nadeem et al., 2016). At the time of parturition, PGRA levels surpass PGRB levels, and functional withdrawal of progesterone due to metabolism by 20αHSD enzyme sequesters PGRB in the cytosol, while unliganded PGRA activates pro-contractile genes to induce labor (Merlino et al., 2007; Nadeem et al., 2016).

PGR gene expression is regulated in a variety of ways including transcriptional regulation via cis- and

trans-acting factors, CpG methylation (Lapidus et al., 1996), histone modifications (Stratmann & Haendler, 2011), mRNA steady-state concentrations (Pei et al., 2018), PGR protein abundance (Lange et al., 2000), and post-transitional modifications (Abdel-Hafiz & Horwitz, 2014). Post-translational modifications play a role in regulating PGR stability and diversify its cellular activity through phosphorylation (Knotts et al., 2001; Lange et al., 2000), sumoylation (Chauchereau et al., 2003), acetylation (Daniel et al., 2010), and ubiquitination (Calvo & Beato, 2011). Trans-acting factors are proteins that bind to specific DNA sequences, known as cis-regulatory elements, to regulate gene expression. Cis-regulatory elements are non-coding DNA regions that regulate the expression of nearby genes; these include promotors that initiate transcription, enhancers that bind TFs to promote gene expression (Field & Adelman, 2020), silencers that bind TFs to decrease gene expression (Petrykowska et al., 2008), and insulators that isolate genomic regions into topologically associated domains (Burgess-Beusse et al., 2002; Yoon et al., 2007).

Progesterone unresponsiveness has been attributed to alterations in PGR isoform expression due to various origins including hypermethylation of PGR regulatory regions (Rocha-Junior et al., 2019; Wu et al., 2006), chronic uterine inflammation (Patel et al., 2017), PGR polymorphisms (Wieser et al., 2002), and changes in gene expression of PGR upstream regulators that have yet to be thoroughly studied (Kim et al., 2015; Lin et al., 2014).

Despite the significant role of progesterone signaling throughout the female reproductive system, much remains unknown about the upstream regulation of both PGR isoforms, including the location of cis-regulatory elements such as enhancers and the identity of trans-acting factors such as transcription factors. The status of histone acetylation marks helps to map the location of potential cis-regulatory elements such as enhancers. Enhancers can be active, marked by the epigenetic marker H3K27ac (Heintzman et al., 2009), or poised, marked by the epigenetic marker H3K27me3 (Rada-Iglesias et al., 2011). Poised enhancers do not regulate gene expression at all times, and instead are cell-type or developmental-stage specific (Rada-Iglesias et al., 2011).

The objective of this study was to identify cis-regulatory elements of PGR isoforms in the myometrium and use these elements to identify novel candidate PGR transcriptional regulators. In this study, candidate regulatory elements of PGR were identified by integrative analysis of H3K27ac marks, Hi-C data, and ATAC-seq data in human myometrial tissues and cells. H3K27ac marks map out the location of potential active enhancer marks, Hi-C data inform on the 3D folding structure of chromatin to delineate what regions of the DNA interact together, and ATAC-seq data identifies regions of the genome that are open and accessible for transcription. The function of candidate regulatory elements was tested using the CRISPR activation (CRISPRa) system. The activation system is comprised of a dead Cas9 enzyme that is mutated at the active site to inhibit its endonuclease activity (Chavez et al., 2015). The dead Cas9 enzyme is fused to a transcriptional activator which is directed by a guide RNA (gRNA) (Chavez et al., 2015). If the gRNA directs the complex to a regulatory element, RNA polymerase is recruited and drives gene expression.

gRNAs targeting around the PGR locus were designed and the single-cell RNA-seq with CRISPR perturbations (Perturb-seq) technology (Datlinger et al., 2017) and RT-qPCR were used to screen the gRNAs in human myometrial cells (hTERT-HM) and human endometrial stromal cells (THESC) to determine whether any gRNAs target regulatory elements of PGR. Results from Perturb-seq and RT-qPCR show that targeting two regions within the vicinity of the PGR locus, the PGR 3' untranslated region (UTR) and an enhancer located 200kbs from the PGR transcription start site (TSS), can successfully induce PGR expression in hTERT-HM cells and THESC. Candidate transcription factors that bind to these regulatory elements were identified using integration of Motif Enrichment and public ChIP-seq data. Motif Enrichment is a bioinformatical approach that identifies transcription factor binding sites that are enriched in a region of the genome and unlikely to be present due to chance, suggesting the potential binding of those transcription factors in that region. On the other hand, public ChIP-seq data is a publicly available database detailing the location of ChIP-determined TF binding sites across various experiments and tissues. Using

the integration of this data, candidate transcriptional regulators that bind to PGR cis-regulatory elements were identified. These include chromatin regulator ARID1A, endometrial epithelial and stromal cell regulators such as GATA2, NR2F2, and ESR1, and myometrial regulators including BRD4 and KLF9. Activation of ESR1 using CRISPRa in hTERT-HM cells results in a significant increase in PGR mRNA abundance.

By uncovering what cis-regulatory elements control the expression of PGR isoforms, we can identify non-coding mutations that lead to altered expression of *PGR*. Through the discovery of PGR regulators, we can improve our understanding of what genetic perturbations underly progesterone resistance. Ultimately, these cis-regulatory elements and transcriptional regulators could be targeted to screen for progesterone resistance or treat the underlying genetic abnormalities that lead to these disease phenotypes.

METHODS

Reagents and cell culture:

Telomerase-transformed human myometrial cells (hTERT-HM) (Condon et al., 2002) and telomerase-transformed human endometrial stromal cells (THESC) (Saleh et al., 2011) were maintained in DMEM/F-12 (Invitrogen, Grand Island, NY, USA) supplemented with 10% Fetal Bovine Serum (FBS, Gibco) and antibiotics (10 000 IU/mL penicillin, 10 000 IU/ mL streptomycin; Life Technologies, Grand Island, NY). Cell culture media was filtered using the 0.22um Rapid-Flow™ Sterile Disposable Filter Units (Nalgene). Cells were cultured and grown in a 5% CO_2 and 37°C incubator. Cells were plated at 20% confluency and the media was changed every three days. Cells were passaged before reaching 90% confluency.

gRNA design and plasmid construction:

To identify candidate proximal and distal regulatory elements of PGR, THESC or hTERT-HM cells were co-transduced with dCas9-VP64-p65-Rta (VPR) and guide RNAs (gRNA) (Chavez et al., 2015). Integration of active enhancer mark, H3K27ac, and HiC chromatin looping in human myometrial tissue and cells was used to identify candidate proximal and distal regulatory regions of PGR. 4-5 gRNAs per region were designed using the CHOPCHOP (Labun et al., 2019) and CRISPick tools (Doench et al., 2016). All gRNA expression vectors were synthesized by and acquired from VectorBuilder (VectorBuilder.com) and expressed neomycin resistance and GFP markers. IGI-P0492 pHR-dCas9-NLS-VPR-mCherry was a gift from Jacob Corn (Addgene plasmid # 102245; http://n2t.net/addgene:102245 ; RRID:Addgene_102245).

Lentivirus production and cell transduction:

To introduce plasmids encoding gRNAs and dCas9-VPR into the cells, viral transduction is performed which uses a lentivirus vector to integrate the plasmid sequences into the cell genome. To make lentivirus, plasmids were isolated from bacteria using Qiagen Plasmid Midi Kit (Qiagen, Valencia, California, USA) according to the manufacturer's instructions. Plasmid quality was assessed by restriction digest and gel electrophoresis. Lentivirus was generated by transfection of the constructs and packaging vector into HEK293T at the NIEHS Viral Vector Core Facility. For viral transduction, cells were seeded at 20% confluency in 10cm plates, 24 hours before transduction. Cells were transduced with gRNA lentivirus at an MOI of 0.4, 2.0, or 4.0 and incubated for 24 hours before washing plates with PBS and replacing them with fresh media. MOI represents the ratio of the number of transducing lentiviral particles to the number of cells, with a higher MOI resulting in more integrations of the plasmid construct into the cells. Cells transduced with gRNA plasmid were selected for using 1mg/ml Geneticin (Gibco, Grand Island, NY) for 3 days (as determined by cell-specific kill curve). Cells were then transduced with dCas9-VPR at an MOI of 4.0, using the same method as above. After transduction with the second plasmid, cells positive for both plasmids were isolated using flow cytometry at the NIEHS Flow Cytometry Center.

Perturb-seq and Data Analysis:

gRNAs targeting candidate regulatory regions of PGR and two control gRNAs were pooled together to generate lentivirus carrying equimolar ratios of each gRNA. THESC and hTERT-HM cells were transduced with gRNA lentivirus at an MOI of 0.4. At an MOI of 0.4,

most cells receive just one gRNA. After transduction with dCas9-VPR at an MOI of 4.0, cells positive for both plasmids were isolated using flow cytometry. The cells received from flow cytometry were counted and examined for viability with trypan blue staining using a TC-20 cell counter (Bio-Rad). Approximately 16,500 live cells at 1×10^6 cells/ml concentration with 65% or above viability were loaded into the Single Cell Chip to generate single cell emulsion in Chromium Controller with Chromium Single Cell 3' Library & Gel Bead Kit v3.1 (10x Genomics, Cat. 1000268). Reverse transcription of mRNA and cDNA amplification were carried out according to the manufacture's instruction (10x Genomics, Cat. 1000268, Cat. 1000262 with 10x Genomics protocol CG000316). The amplified cDNA was separated into CRISPR sgRNA derived cDNA and transcriptome derived cDNA. The CRISPR sgRNA derived cDNA was used to make NGS sequencing libraries. The transcriptome derived cDNA was further fragmented to construct NGS libraries. Both libraries were then sequenced together with the molar ratio of 1 to 4 by the NIEHS Epigenomics and DNA Sequencing Core Laboratory according to the manufacture's instruction.

Raw data from the Perturb-seq assay was processed and analyzed at the NIEHS bioinformatics core. Briefly, the raw sequencing FASTQ files generated from both the transcriptome and CRISPR screening libraries were processed together by Cell Ranger software (version 4.0.0, 10× Genomics). The "cellranger count" pipeline used STAR for aligning the reads to the human reference, GRCh38 "refdata-gex-GRCh38-2020-A" (10X Genomics), and associated gene expression profile with gRNA identity by a unique barcode in each cell. Seurat software (version 3.6.3) was utilized to perform clustering analysis on the combined dataset (Satija 2015, PMID: 25867923). The SCTransform package was applied to normalize gene expression counts across cells (Hafemeister 2019, PMID: 31870423). The cells were clustered based on the number of unique gRNAs detected. Cell populations containing more than one unique gRNA were excluded from further analyses. The expression level of PGR in each cell containing the same gRNA was compared to cells containing the non-targeting control gRNA. A fold-change (FC) greater than 1.5 in PGR mRNA levels was defined as significant.

RT-qPCR assay:

In order to test for PGR expression in the transduced cells, RT-qPCR was employed. RNA was isolated from cells using the RNeasy mini RNA isolation kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. 0.4 ug of RNA was reverse transcribed into complementary DNA (cDNA) using Moloney Murine Leukemia Virus reverse transcriptase (Thermo Fisher Scientific) with Random Hexamers (Invitrogen, Waltham, MA, USA) according to manufacturer protocol. For quantitative analysis of mRNA, SsoAdvanced Universal SYBR Green Supermix (BioRad, Hercules, CA, USA) was used according to manufacturer instructions. Each reaction was performed in technical duplicates using the standard curve-based method (Larionov et al., 2005). Briefly, reaction samples were prepared to a total volume of 20ul with 5uM of each of the forward and reverse primers **(Table 1.1)**, 4ul of cDNA, and a final 1x concentration of the SYBR Green Supermix. Standards were made by pooling together 6ul of cDNA from each sample and diluting to 140ul by adding DNase-free water. Pooled cDNA was then diluted in DNase-free water to final relative concentrations of 100%, 25% and 10%. The reaction was heated to 95°C for 30 seconds, followed by 39 cycles of denaturation at 95°C for 15 seconds and annealing and elongation at 60°C for 30 seconds. Temperature cycles were performed on the CFX Connect™ Real-Time PCR Detection System (Bio-Rad). Relative mRNA levels of PGR were compared to the negative control (non-targeting control) and positive control (PGR640A).

Table 1.1: Primers used for RT-qPCR

PGR	Forward	CTGGCATGGTCCTTGGAG
	Reverse	TCATTTGGAACGCCCACT
ESR1	Forward	CTGCAGGGAGAGGAGTTTGT
	Reverse	TCCAGAGACTTCAGGGTGCT
18S	Forward	GTAACCCGTTGAACCCCATT
	Reverse	CCATCCAATCGGTAGTAGCG

These primers were used in RT-qPCR to target the mRNA transcript of PGR, ESR1, and the housekeeping gene 18S to confirm upregulation of these genes by CRISPR activation.

Generating ESR1 expressing hTERT-HM cells:

To activate ESR1 expression, gRNAs were designed to target the promotor of the ESR1 gene using the same methods and tools as above. Plasmids were designed and lentivirus was produced as mentioned above. hTERT-HM cells were transduced with gRNA at an MOI of 4.0. Cells transduced with gRNA plasmid were selected for using 1mg/ml Geneticin (Gibco, Grand Island, NY) for 3 days. Cells were then transduced with dCas9-VPR at an MOI of 4.0, using the same method as above. After transduction with the second plasmid, cells positive for both plasmids were isolated using flow cytometry at the NIEHS Flow Cytometry Center. RT-qPCR and western blot were conducted to measure the levels of ESR1 mRNA and protein in cells.

Western Blot:

A western blot was employed to determine whether ESR1 was upregulated due to transduction. To isolate protein from cells, the RIPA Lysis and Extraction Buffer protocol was followed (Thermo Scientific) with the following specifications/modifications: approximately 300,000 cells were pelleted and lysed with 100ul of complete Pierce RIPA buffer. Protein was quantified using the BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA) as instructed by the manufacturer. Western blot was conducted with 40ug of protein per sample. Protein lysates from MCF7 cells were used as positive controls, while protein lysates from HEK293T cells were used as negative controls. Western blots were run on Mini-PROTEAN TGX Pre-cast Protein gels (Bio-Rad, #4568094) with the Precision Plus Protein Dual Color Standards Ladder (Bio-Rad #1610374). Gels were transferred to nitrocellulose membrane using the Turbo-Blot transfer system (BioRad) according to the manufacturer's directions. The membrane was blocked with 5% milk (Santa Cruz Biotech, Santa Cruz, CA, USA) in TBST (20 mM Tris, pH 7.4 (Lonza, Morrisville, NC, USA), 140 mM NaCl (Lonza), 1% TWEEN-20 (Sigma) (Hewitt et al., 2022). ESR1 protein was detected by incubating with ESR1 specific antibodies **(Table 1.2)** diluted 1:1000 in 5% milk overnight at 4C. Bands were detected with secondary antibodies **(Table 1.2)** diluted 1:20,000 in 5% milk for 45 minutes and imaged using Odyssey Fc Imager (LI-COR Biosciences) using 800nm channel for 10 minutes, and 700nm channel for 30 seconds (to image ladder). The control protein (B-actin) was detected similarly as above with the antibodies and dilutions included in the table below.

Table 1.2: Antibodies used for Western Blot

Target Protein	Antibody	Dilution
ESR1	**Primary:** ERa (H-184) sc-7207, Lot#G2109 rabbit polyclonal IgG Santa Cruz	1:1000
	Secondary: Goat anti-rabbit 926-32211 Lot#DOO304-15.	1:20000
B-Actin (control)	**Primary:** Actin (I-19)-R sc-11616-R, Lot#DO406 Rabbit polyclonal IgG Santa Cruz.	1:50000
	Secondary: Goat anti-rabbit 926-32211 Lot#DOO304-15.	1:20000

These primary antibodies were used in western blot to detect ESR1 protein and the housekeeping control protein B-Actin to confirm upregulation of these genes by CRISPR activation. The secondary antibodies bind to the primary antibodies for visualization using fluorescence.

Identifying candidate PGR upstream regulators:

To identify candidate PGR upstream regulators, motif enrichment (JASAPR) and public ChIP-seq data (ReMap) were used to screen for potential transcription factors (TFs) that bind to the *PGR* enhancer. All TFs that either had enriched motifs in the enhancer (JASPAR) or were found to bind to the enhancer (ReMap) in at least one study were considered. The TF gene list was narrowed down by identifying genes with translated protein products present in the endometrium or myometrium of human biopsies using data from the Human Protein Atlas (Uhlen et al., 2015). If the protein product of the gene was not detected in myometrium or endometrium biopsies, it was eliminated. Next, candidate PGR regulators were further narrowed down by identifying TF genes with reproductive or embryonic lethal phenotypes in mouse knock-out models using data from MGI Jax. Only reproductive phenotypes in female mice were considered, which included anything related to decreased fertility or litter size, and abnormal uterus morphology. Lastly, a literature search was conducted to further narrow down the gene list; genes that have been discovered by previous studies to play important roles in the myometrium or endometrium or involved with endometriosis, decidualization, or parturition/labor were selected.

RESULTS

Identification of putative proximal and distal regulatory elements of PGR.

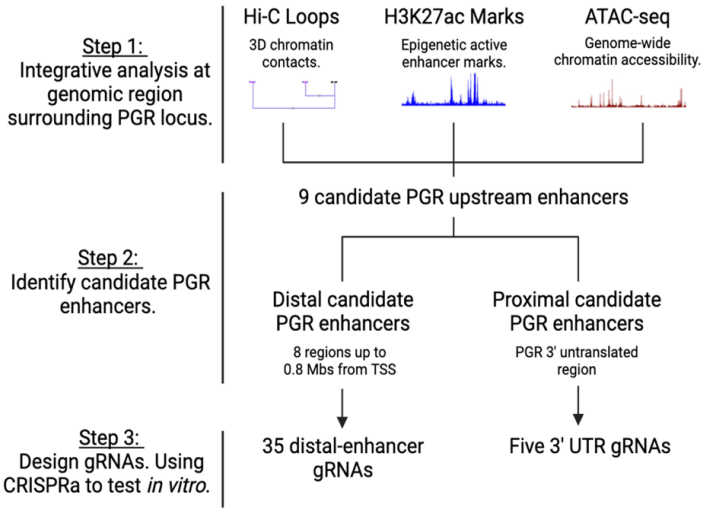

Figure 1: Workflow to identify and test candidate PGR enhancers.

To identify candidate cis-regulatory regions of *PGR*, integrative analysis of H3K27ac data, ATAC-seq data, and Hi-C looping data was used **(Figure 1)**. The following criteria were used to identify the candidate regions:

1. The presence of the active enhancer marks H3K27ac (Ji et al., 2015) in myometrial biopsy samples and the absence of H3K27ac

marks and ATAC-seq peaks in *in vitro* cultured myometrial cells. Upon the removal of myometrial cells from the body and culture *in vitro*, there is a rapid reduction in *PGR* expression levels. Epigenetic data indicates that there is a loss of H3K27ac marks in cultured myometrial cells at distinct regions around the PGR locus, in comparison to myometrial biopsies (unpublished observations). The loss of these marks at PGR enhancers promote chromatin condensing and gene silencing and may be responsible for this decrease in PGR expression.

2. The presence of chromatin interactions between the *PGR* promotor and distal regions of the genome, as identified by Hi-C data. Hi-C data identifies regions of chromatin that loop together, which allows regulatory elements residing within the loops to be brought closer together to associate with each other topologically for the regulation of gene expression (Palstra, 2009). It was arbitrarily defined that enhancer elements present in regions up to three connections away from the *PGR* transcription start site (TSS) will be considered for this study, covering a 1 Mb around the PGR TSS. Studies have shown that most enhancers lay within 1 Mb upstream or downstream of the gene TSS (Mora et al., 2016).
3. Significant ReMap peak. ReMap is a database of publicly available ChIP-seq data detailing the location of transcription factor (TF) binding sites across various experiments and tissues (Hammal et al., 2022). A significant ReMap peak indicates that a particular region of the genome is a TF hotspot, suggesting that it could be a regulatory domain.

Using these criteria, nine putative regulatory elements of PGR were defined, six regions upstream of the *PGR* TSS (denoted U1-6) and three regions downstream of the *PGR* TSS (denoted D1-3) **(Figure 2)**. The D1 region targets the 3' untranslated region (UTR) of *PGR*. 4-5 gRNAs were designed to target different locations within each region to ensure that each region has at least two working gRNAs, resulting in a total of 40 gRNAs. gRNAs were divided into two groups, a 3' UTR proximal gRNA pool with 5 gRNAs and a Distal-Enhancer gRNA pool with the remaining 35 gRNAs **(Figure 1)**.

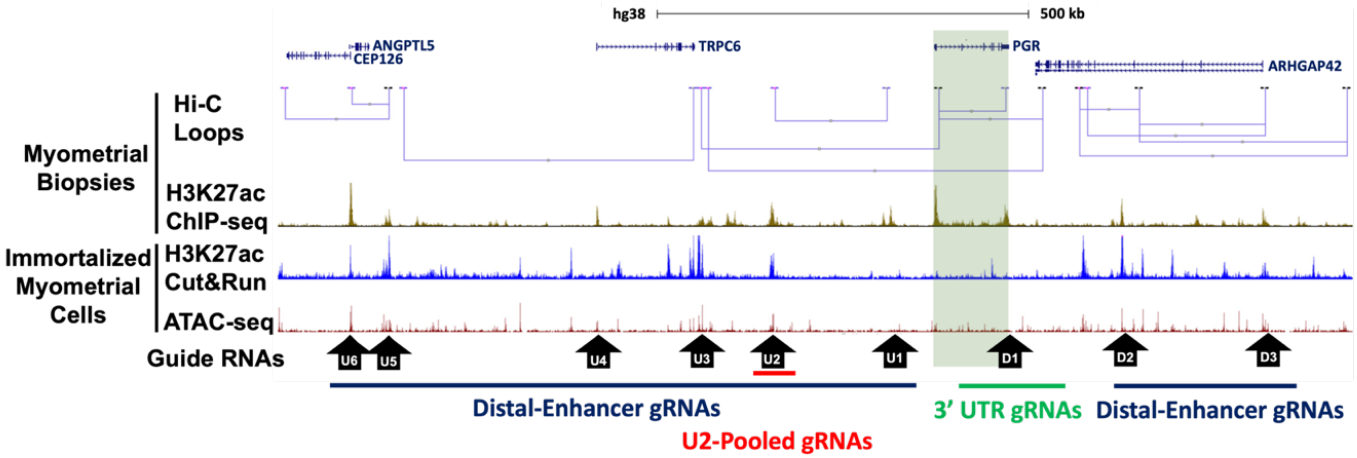

Figure 2: Integrative analysis for identification of putative proximal and distal regulatory elements of PGR. UCSC Genome Browser track view of the human PGR locus (highlighted in green) and surrounding regions marked with gRNA targeting locations (black arrows). Locations of gRNAs targeting distal-enhancer regions (blue) and 3' UTR (green) are highlighted.

Targeting distal PGR putative enhancers in uterine cells by RT-qPCR and Perturb-seq.

To test whether any gRNAs successfully target distal cis-regulatory elements of *PGR*, the Distal-Enhancer gRNA pool was introduced into uterine cells and *PGR* mRNA levels were measured with RT-qPCR. Transduction of hTERT-HM cells with dCas9-VPR and the Distal-Enhancer gRNA pool at a multiplicity of infection (MOI) of 4 resulted in a 1.89-fold-change (FC) of *PGR* mRNA levels (P-value < 0.05) **(Figure 3A)**. MOI refers to the ratio of viral transducing particles to cells. Transduction of THESC with dCas9-VPR and the Distal-Enhancer gRNA pool at an MOI of 4 resulted in a 1.88-FC of *PGR* mRNA levels (P-value < 0.05) **(Figure 3B)**. These results suggest that some of the gRNAs in the Distal-Enhancer gRNA pool can activate PGR expression, presumably by targeting cis-regulatory elements, and therefore, Perturb-seq was conducted to screen for functional cis-regulatory elements of *PGR* (Datlinger et al., 2017).

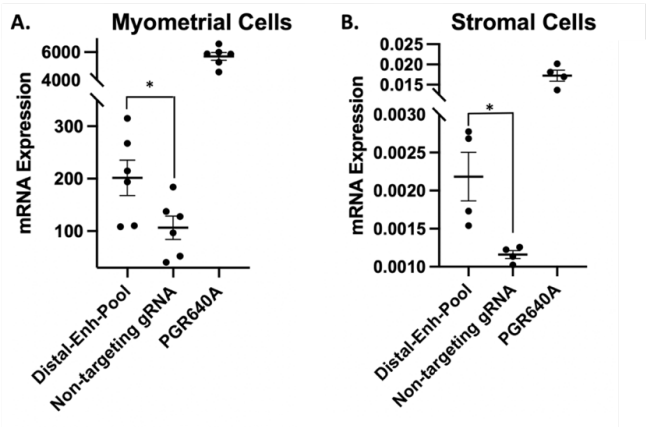

Figure 3: Targeting candidate PGR distal enhancers by CRISPR activation in mesenchymal lineage cells. Relative PGR mRNA levels measured by qRT-PCR in hTERT-HM cells **(A)** or T-HESC **(B)** that express denoted gRNAs with the CRIPSR activator, dCas9-VPR. N = 3 with technical duplicates **(A)**, N = 2 with technical duplicates **(B)**. Relative PGR mRNA levels were compared to PGR-promoter targeting (PGR640) or non-targeting gRNAs as controls. *P-value < 0.05 by unpaired t-test.

The perturb-seq assay combines CRISPR-Cas9 based gene perturbation and single-cell RNA sequencing to study the effects of specific genetic perturbations on gene expression in individual cells at a large scale (Datlinger et al., 2017). By identifying the gRNA introduced into individual cells and the level of *PGR* mRNA in those cells, Perturb-seq permits the identification of specific gRNAs that result in a significant upregulation of *PGR* mRNA compared to cells receiving the non-targeting control gRNA. For this study, significant upregulation was defined as an mRNA level FC greater than 1.5 which follows conventional practices. Both the Distal-Enhancer gRNA pool and 3' UTR gRNA pool were introduced into uterine cells hTERT-HM and THESC at a total MOI of 0.4, resulting in the majority of cells receiving a single gRNA. Using the transcriptome of each individual cell as a data point, the goal is to collect 7,000 data points per assay for an average of 100 data points for each individual gRNA to secure statistical power.

hTERT-HM cells transduced with both the Distal-Enhancer gRNA pool and 3' UTR gRNA pool and subjected to Perturb-seq did not yield any significant results, with no gRNAs resulting in a significant upregulation of *PGR* mRNA in comparison to the control. A total of 132 individual cells containing gRNA-21 were tested with an average *PGR* mRNA $\log_2$FC of 1.20 in comparison to non-targeting control (P-value = 0.03) **(Figure 4A)**. Perturb-seq in THESC identified significant upregulation of *PGR* mRNA in cells expressing gRNA-21 and gRNA-22 in comparison to the non-targeting control **(Figure 4B)**. A total of 102 individual cells containing gRNA-22 were tested with an average *PGR* mRNA $\log_2$FC of 2.00 in comparison to non-targeting control (P-value = 2.21×10^{-10}) **(Figure 4A)**. gRNA-21 and 22 correspond to gRNAs targeting the U2 putative enhancer region. To validate these results, RT-qPCR was conducted in hTERT-HM cells transduced with 5 pooled gRNAs targeting the U2 enhancer region (including gRNA-21 and 22). Transduction with these gRNAs resulted in a significant upregulation of *PGR* mRNA with a FC of 5.57 (P-value < 0.001) in comparison to non-targeting control **(Figure 4B)**.

A.

gRNA	Fold Change over Non-targeting Control (log2)	*P-value*	N
gRNA-21	1.20	0.03	132
gRNA-22	2.00	2.21×10^{-10}	102
Positive Control	3.17	2.70×10^{-35}	148

B.

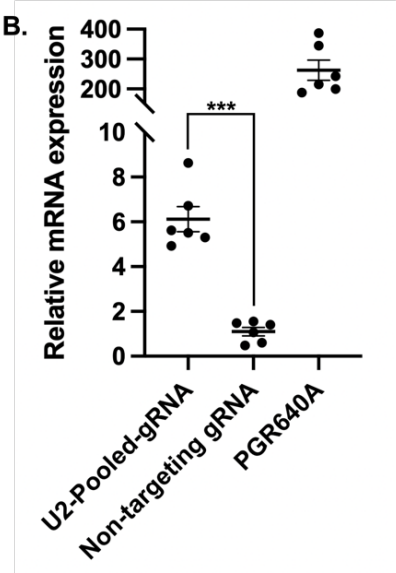

Figure 4: Using Perturb-seq to screen for candidate PGR distal enhancers.
(A) Relative PGR mRNA levels measured by RNA-seq in THESC cells that express denoted gRNAs with the CRIPSR activator, dCas9-VPR. Log2(FC) compares relative mRNA abundance in experimental samples over non-targeting control. N indicates the number of cells used for statistical analysis. **(B)** Relative PGR mRNA levels measured by qRT-PCR in hTERT-HM cells that express denoted gRNAs with the CRIPSR activator, dCas9-VPR (N = 3 with technical duplicates). *P-value < 0.05, **P-value < 0.01, ***P-value < 0.001, ****P-value < 0.0001 by unpaired t-test.

Targeting the *PGR* 3' UTR in myometrial cells by RT-qPCR.

Studies have shown that strong H3K27ac marks are conserved at the *PGR* 3' UTR in both human and mouse uterine tissues, suggesting a functional role of this region (Li et al., 2021; Wu et al., 2022). Previous experiments in the lab have demonstrated concern over the lower sensitivity of Perturb-seq in comparison to manual RT-qPCR screening (unpublished observations). Additionally, there is Hi-C looping between the *PGR* promotor and 3' UTR, suggesting that these regions interact to regulate gene expression through activation or inhibition. There is also a significant reduction in H3K27ac marks and ATAC-seq signal at the *PGR* 3' UTR in myometrial cells in comparison to tissue, a possible reason for the downregulation of PGR in myometrial cells in comparison to tissue. The limited sensitivity of the Perturb-seq assay, alongside the FC cut-off, may have caused the regulatory activity of the *PGR* 3' UTR to be missed. Therefore, it was selected for manual screening.

In order to determine whether the *PGR* 3' UTR contains a *PGR* cis-regulatory sequence, hTERT-HM cells were transduced with dCas9-VPR and the 3' UTR gRNA pool, and *PGR* mRNA levels were determined by RT-qPCR. Transduction of hTERT-HM cells resulted in significant upregulation of *PGR* mRNA levels in a dose-dependent manner **(Figure 5A)**. At the lowest MOI of 0.4, *PGR* mRNA was upregulated 1.98-fold (P-value < 0.01), at an MOI of 2, PGR mRNA was upregulated 2.94-fold (P-value < 0.0001), and at an MOI of 4, *PGR* mRNA was upregulated 3.51-fold (P-value < 0.0001) **(Figure 5A)**. These results indicate that at least one of the gRNAs in the 3' UTR gRNA pool targets a cis-regulatory element of *PGR*. In order to determine which gRNAs in specific from the 3' UTR gRNA pool upregulate *PGR* mRNA levels, hTERT-HM cells were transduced with three individual gRNAs from the PGR 3' UTR gRNA pool. Transduction with each of these gRNAs resulted in significant upregulation of *PGR* mRNA, with gRNA D1D having the most significant upregulation with a FC of 2.88 (P-value < 0.0001) **(Figure 5B)**.

Identifying potential PGR upstream regulators that bind to PGR 3' UTR.

The identification of a *PGR* enhancer at the *PGR* 3' UTR opens the possibility of identifying upstream regulators of PGR that bind to this enhancer. Motif enrichment (JASAPR) and public ChIP-seq data (ReMap) were used to screen for potential TFs that bind to the *PGR* 3' UTR **(Figure 6)**. Candidate PGR regulators were first narrowed down by identifying genes that had lower levels of PGR expression in hTERT-HM cells in comparison to human myometrial biopsies using RNA-seq data from

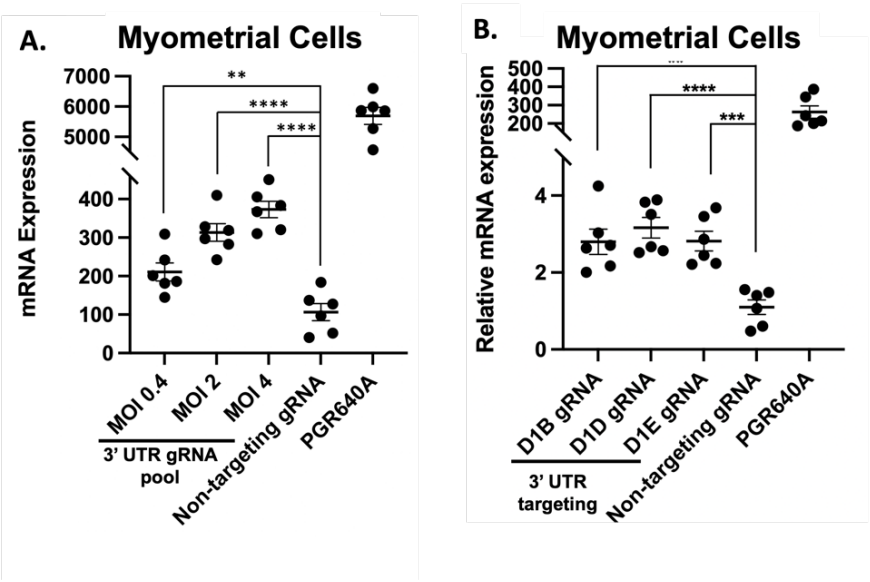

Figure 5: Targeting PGR 3' UTR by CRISPR activation in mesenchymal lineage cells. Relative PGR mRNA levels measured by qRT-PCR in hTERT-HM cells that express denoted gRNAs with the CRIPSR activator, dCas9-VPR (N = 3 with technical duplicates). Cells were transduced with gRNA at an MOI of 0.4, 2 and 4 **(A)** or MOI of 4 **(B)**. Relative PGR mRNA levels were compared to PGR-promoter targeting (PGR640) or non-targeting gRNAs as controls. **P-value < 0.01, ***P-value < 0.001, ****P-value < 0.0001 by unpaired t-test.

myometrial cells and tissue (PMID: 31908010 and unpublished observations). There were two reasons for this: (1) the hTERT-HM cells used in this study have lower levels of *PGR* mRNA in comparison to human myometrium biopsies, suggesting the possibility that upstream activators of PGR may be downregulated in hTERT-HM cells in comparison to tissue, and (2) the CRISPR activation assay has been thoroughly established in this cell line and will therefore be the main tool used to screen for candidate activators of PGR in subsequent studies. The TF gene list was further narrowed down by identifying genes with translated protein products present in the endometrium or myometrium of human biopsies using data from the Human Protein Atlas (Uhlen et al., 2015) **(Figure 6)**. If the protein product of the gene was not detected in the myometrium or endometrium biopsies, it was eliminated. Next, candidate PGR regulators were further narrowed down by identifying TF genes with reproductive or embryonic lethal phenotypes in mouse knock-out models using data from MGI Jax, implicating a major biological role for these genes in the uterus **(Figure 6)**. Lastly, a literature search was conducted to further narrow down the gene list; genes that have been discovered by previous studies to play important roles in the myometrium or endometrium or involved with endometriosis, decidualization, or parturition/labor were selected. The following genes were identified as potential activators of PGR in the endometrium: GATA2, FOXP1, ARID1A, NR2F2, ESR1, and MED1. The following genes were identified as potential activators of PGR in the myometrium: BRD4, MED12, NFATC, KLF9, and ESR1 **(Figure 6)**.

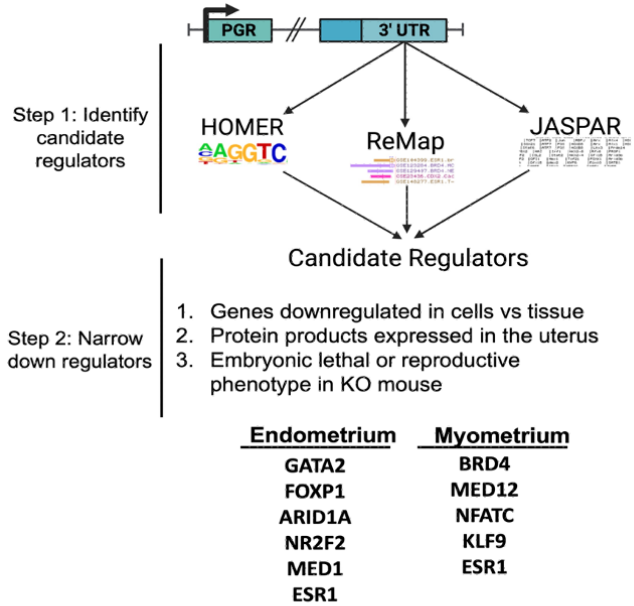

Figure 6: Strategy to identify PGR upstream regulators that act through the PGR 3'UTR in uterine mesenchymal cells.

Testing the effect of ESR1 expression on PGR mRNA levels in myometrial cells.

Because ESR1 is a known upstream regulator of *PGR* in the endometrium (Chantalat et al., 2020), the effects of its expression on *PGR* mRNA levels *in vitro* were tested. The use of ESR1 serves as a proof of principle on the methodology for future studies on the mechanism of action of ESR1 regulation of *PGR* transcription. Two gRNAs targeting the ESR1 promotor (ESR1-3 and ESR1-6) were introduced into hTERT-HM cells alongside dCas9-VPR. Western blot shows that hTERT-HM cells transduced with ESR1-3 gRNA express the ESR1 protein (66-kDa) in comparison to the unmanipulated positive control MCF7, a human breast cancer cell line (Comsa et al., 2015; Soule et al., 1973) **(Figure 7)**. A prominent band detected by the ESR1 antibody at roughly 46-kDa may be an ESR1 variant (Staub et al., 2005) **(Figure 7)**. Treatment of ESR1-expressing cells with the estrogen ligand Estradiol at a concentration of 10nM for 6 hours resulted in significant upregulation of *PGR* mRNA in hTERT-HM cells with a FC of 4.80 (P-value < 0.01) in comparison to non-targeting

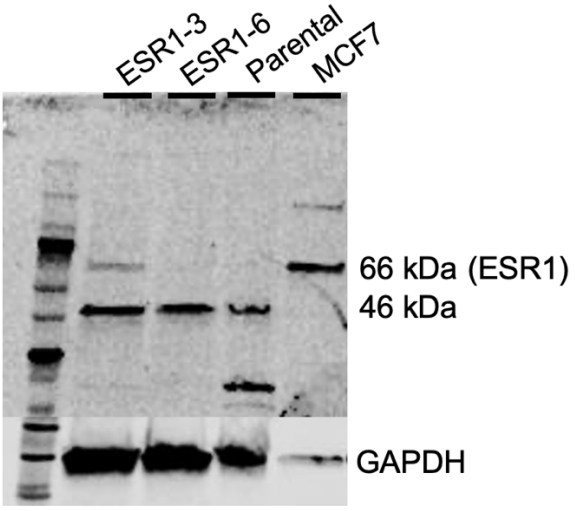

Figure 7: Myometrial cells genetically engineered to express ESR1.
Western Blot confirms the upregulation of ESR1 protein (66 kDa) in hTERT-HM cells expressing ESR1-3 gRNA alongside the CRISPR activator, dCas9-VPR. Protein levels from unmanipulated hTERT-HM cells and MCF7 cells serve as negative and positive controls for ESR1 presence. GAPDH is the loading control.

control **(Figure 8B, D)**. This finding supports ESR1 as an upstream regulator of *PGR*.

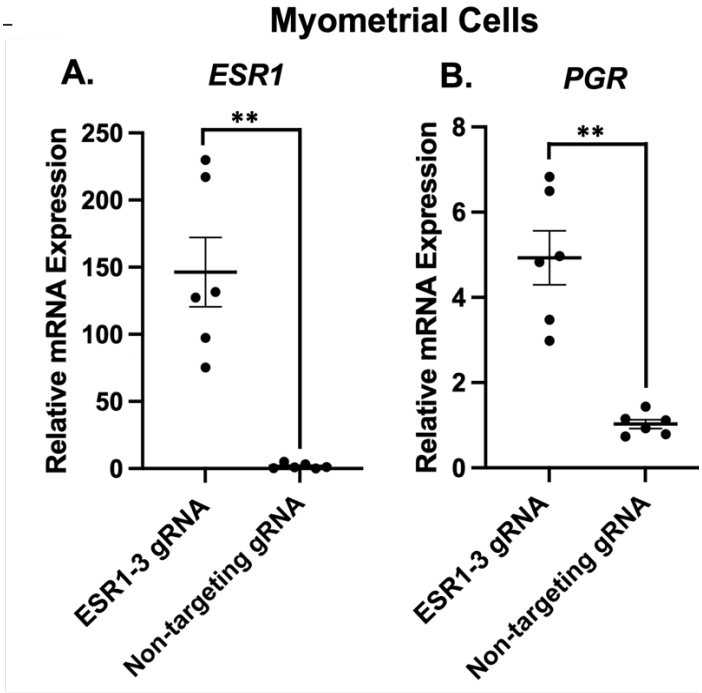

Figure 8: Testing the effect of PGR regulator, ESR1, in mesenchymal lineage cells.
Relative ESR1 **(A)** and PGR **(B)** mRNA levels measured by qRT-PCR in hTERT-HM cells that express denoted gRNAs with the CRIPSR activator, dCas9-VPR (N = 3 with technical duplicates), treated with 10 nM Estradiol for 6 hours. **(B)** Relative PGR mRNA levels were compared to PGR-promoter targeting (PGR640) or non-targeting gRNAs as controls. **P-value < 0.01, ***P-value < 0.001, ****P-value < 0.0001 by unpaired t-test.

DISCUSSION

PGR expression is regulated in a tissue and cell-specific manner via cis- and trans-acting factors, playing key roles in both the myometrium and endometrium for the regulation of embryo implantation, decidualization, parturition, and menstruation. Previous studies demonstrated that hypermethylation at regulatory regions around the PGR locus leads to altered PGR expression and progesterone resistance, resulting in impaired embryo implantation and

decidualization (Su et al., 2016). Through integrative analysis and CRISPR activation, this study identifies two new PGR enhancers located at the *PGR* 3' UTR (Region D1) and 200kbs upstream of the *PGR* TSS (Region U2). The identification of these enhancers revealed potential upstream regulators that control PGR isoform expression in the uterus, including GATA2, ARID1A, and KLF9. This study confirms that ESR1 gene expression is able to induce PGR expression in endometrial stromal cells, supporting previous studies. Moreover, this study shows that ESR1 expression is also able to induce PGR expression in myometrial cells, creating a new avenue to investigate the regulatory relationship between ESR1 and PGR in the myometrial compartment of the uterus.

Results from motif enrichment identified ESR1 as the most abundant TF binding motif in the *PGR* 3' UTR. ESR1 has been widely studied for its role in the endometrium, including signaling for embryo implantation and decidualization, as well as for its mis-regulation in patients with endometriosis (Chantalat 2020). For these reasons, the effect of liganded ESR1 on *PGR* mRNA levels was tested first as a proof of principle on the methodology. Furthermore, identifying ESR1 here supports the validity of the screening strategy in finding plausible targets. It has been shown that estrogen, the ESR1 ligand, acts as an upstream regulator of PGR in the uterus (Wendell 1971) by binding to estrogen response elements located in the regulatory regions of *PGR* (Savouret, 1991). After ovulation, estrogen acting through ESR1 in the endometrial stroma promotes the proliferation of endometrial epithelial cells and activates expression of PGR (Furuminato 2023). Subsequently, progesterone binding to PGR represses estrogen induced proliferation of epithelial cells, allowing for embryo implantation during the window of receptivity (Furuminato 2023). The effects of estrogen and ESR1 on *PGR* expression is dependent on co-regulators, such as SRC-1, allowing ESR1 to have both activation and inhibitory action on *PGR* expression (Oñate 1995). The results from this study support the fact that liganded ESR1 upregulates *PGR* mRNA expression in the stromal compartment of the uterus. The effects of estrogen and ESR1 on *PGR* expression in the myometrium have been much less studied. The results of this study show that in the myometrium, liganded ESR1 can also upregulate *PGR* mRNA levels.

Other candidate upstream PGR regulators identified through integrative analysis that bind to the *PGR* 3' UTR include key uterine regulators *GATA2*, *ARID1A*, and *KLF9*. Ablation of these genes in the mouse uterus results in infertility by interfering with key processes including embryo implantation, stromal decidualization (Rubel et al., 2016) (Kim et al., 2015), and parturition (Zeng et al., 2008). At the start of pregnancy, *GATA2* expression closely mirrors that of *PGR* expression (Rubel et al., 2012), and GATA2 ablation in the mouse uterus results in a significantly decreased expression of both PGRA and PGRB (Rubel et al., 2016), suggesting a potential activating role of GATA2 on *PGR* gene expression for pregnancy establishment. ARID1A encodes for a SWI/SNF subunit that plays a role in chromatin remodeling by breaking DNA-histone contacts (Mathur, 2018). ARID1A knock-out mice have a significant reduction in H3K27ac marks at the PGR locus, as well as a decrease in *PGR* expression levels (Asaka et al., 2023), suggesting epigenetic regulation of PGR by ARID1A through chromatin accessibility. *KLF9* expression has been reported in both the myometrium and endometrium compartments of the uterus (Pabona et al., 2015; Zeng et al., 2008). Significant reductions in both KLF9 and PGR levels in the endometrium have been recorded in patients with endometriosis (Pabona et al., 2015). These results alongside the results of this study strongly indicate a potential role of these genes in the regulation of *PGR* isoform expression in the uterus, and potentially in the rise of progesterone resistance phenotypes.

Future Studies:

The impact of these candidate regulators on *PGR* gene expression will be tested by activating their expression through CRISPR activation and looking at resulting changes in PGR mRNA levels through RT-qPCR and PGR isoform levels through western blot. The identified *PGR* regulators will be further examined for their genome occupancy in the *PGR* locus and the associated enhancers by the chromatin immunoprecipitation assay. Gain and loss of function assays of the identified *PGR* regulators will be carried

out in multiple lines of primary uterine cells to validate their functionality on *PGR* expression control.

Limitations:

With the use of the Perturb-seq assay, a PGR enhancer located 200kbs upstream of the *PGR* TSS was identified; however, the Perturb-seq assay was unable to detect the PGR cis-acting element at the 3' UTR which was identified through RT-qPCR. This data demonstrates a trade-off between efficiency and extensiveness of the Perturb-seq assay in comparison to manual RT-qPCR; with the sensitivity of sc-RNA-seq being insufficient as a comprehensive assay to survey all cis-acting elements in the vicinity of a gene. Furthermore, while the Perturb-seq assay was able to identify enhancers in stromal cells (THESC), no enhancers were identified in myometrial cells (hTERT-HM). This may be due to cell type specific changes in epigenomic signals at the *PGR* locus in myometrial cells in comparison to endometrial cells. The *PGR* promotor contains a CpG methylation site which has been shown to silence *PGR* expression due to hypermethylation, resulting in impaired embryo implantation and decidualization (Su et al., 2016). Furthermore, myometrial cells have a significant reduction in H3K27ac marks at the PGR promotor in comparison to myometrial tissue **(Figure 2)**, a likely factor contributing to the low gene expression of *PGR* in myometrial cells. It is possible that DNA methylation or histone deacetylation at the *PGR* promotor interfere with the induction of *PGR* expression by *PGR* enhancers, falling below the detection threshold for Perturb-seq. In order to boost up the signal-to-noise ratio, a stronger transcriptional activator than VPR may be used, such as SPH (Zhou et al., 2018) or CBP (Sajwan & Mannervik, 2019). Furthermore, two or more enhancers could be targeted simultaneously, or enhancers could be targeted in conjunction with the gRNA that targets the *PGR* promotor, allowing for the identification of more sensitive enhancers.

Though epigenome data from myometrial tissue was used to infer the location of putative upstream regulatory elements, this study exclusively relied on results from immortalized cell lines to test the activity of these regulatory elements. Due to the artificial conditions maintaining these cell lines, these cells may only offer a narrow window to capture *in vivo* biology. In the future, these studies will be replicated in multiple primary cell lines to validate the functionality of the enhancers on *PGR* expression control.

This study screened nine candidate enhancer regions located up to 600kbs away from the *PGR* TSS. The mechanism by which promotors communicate with distant enhancers, also known as "enhancer-promoter communication", has not yet been elucidated. One hypothesis is the looping model that postulates that enhancer-promotor communication occurs through chromatin loops that bring these elements within close proximity through the formation of topologically associated domains (Razin et al., 2023). Based on the looping model, this study arbitrarily identified putative enhancer regions up to three loop connections away from the *PGR* TSS. However, previous studies have shown that enhancers can regulate genes located up to 1Mb away, as seen for the SHH gene (Lettice et al., 2003), highlighting the limited scope of this experiment. Furthermore, due to the lower sensitivity of Hi-C looping data in tissue, key chromatin loops that inform on DNA interactions may have been missed.

Impact

Cis-regulatory elements and upstream transcription factor regulators play a pivotal role in the precise orchestration of gene expression and downstream gene networks. Cis-regulatory elements such as enhancers act as fine tuners for gene expression, allowing genes to respond to internal and external stimuli, such as the physical contact between the embryo and endometrium that triggers a cascade of gene expressions, allowing the uterus to support pregnancy. Understanding the regulatory elements of a gene is fundamental to deciphering the complex regulatory networks that sustain normal cellular function and development, as well as what goes wrong when disease phenotypes arise. Many disease phenotypes arise from mutations in the regulatory sequence of genes rather than the coding sequence, which can lead to alterations in the expression of a gene, rather than its translated protein product. For example, mis-regulation of PGR isoforms can alter the PGRB:PGRA ratio, a factor present in uterine pathologies including endometriosis, preterm birth,

and progesterone resistance (Mousazadeh et al., 2019; Nothnick, 2022; Peavey et al., 2021). By uncovering what cis-regulatory elements control the expression of PGR isoforms, we can identify non-coding mutations that lead to altered expression of *PGR*. Furthermore, through the discovery of upstream regulators of PGR, we can begin to decipher the underlying cause of altered *PGR* expression, and potentially, what gives rise to progesterone resistance. Ultimately, these regulators could be targeted to screen for progesterone resistance or treat the underlying genetic abnormalities that lead to these disease phenotypes.

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Exploring Serotonin in Catch Contractions and Specific Dynamic Action in Postprandial Metabolism: Insights from Aquatic Models

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Article Synopsis

Animal model organisms have long been instrumental in unraveling complex biological processes relevant to human physiology. The first study investigates how exercise and food intake affect metabolism, using zebrafish as our model. The second experiment dives into how neurotransmitters like dopamine and serotonin control muscle movements, particularly catch contractions, in mussels. Examining these processes provides insights into how muscle function and metabolism are regulated, potentially shedding light on human health and conservation efforts.

Graphic by Erin Heyeck

Exploring Serotonin in Catch Contractions and Specific Dynamic Action in Postprandial Metabolism: Insights from Aquatic Models

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Preface

By investigating the biological processes using aquatic models such as mussels and zebrafish, new pathways can be explored to better understand fundamental aspects of physiology and behavior. In the following chapters, two studies are presented that exemplify the significance of employing aquatic organisms in scientific inquiry. These studies not only provide valuable insights into the specific mechanisms like metabolism and muscle contractions but also offer broader implications for our understanding of human physiology and ecological conservation.

The first chapter explores the relationship between exercise, food intake, and metabolic rate, employing zebrafish as a model organism. This exploration centers on specific dynamic action, a phenomenon that suggests metabolic rate increases following food consumption. It is hypothesized that digestive processes, such as enzyme secretion and nutrient transportation, incur energetic costs, thereby boosting overall energy expenditure. However, the precise extent of these energetic expenditures and the breadth of species affected remain poorly understood. Through the investigation of the impacts of exercise and feeding on metabolic rate, this research aims to better understand the extent of specific dynamic action and its ramifications for energy utilization. In doing so, it offers valuable insights into animal feeding and husbandry practices while providing insight into the energetic demands of digestion.

In the second chapter, the neurobiology of muscle control is investigated, focusing on neurotransmitters such as dopamine and serotonin, utilizing mussels as the model organism. Mussel catch contractions represent a unique physiological adaptation that allows these organisms to sustain muscle contractions for extended periods with minimal energy expenditure, protecting themselves against predation. This mechanism relies on the modulation of neurotransmitters, which signal initiation and relaxation of the neuromuscular contraction pathway. This investigation aims to unravel the intricate neural circuits and signaling pathways involved in modulating muscle function. The study of mussel catch contractions not only unveils the fascinating adaptations of these organisms but also holds promise for biomimetic engineering applications and enhances our understanding of muscle physiology.

Together, these studies underscore the invaluable role of aquatic model organisms in advancing our understanding of physiology, health, and conservation. By leveraging the unique attributes of these organisms, these experiments not only unravel the mysteries of the natural world but also pave the way for innovative discoveries with broad applications.

INTRODUCTION

Studying fundamental physiological processes through model organisms provides a unique window into understanding mechanisms that underpin broader biological phenomena. Two such processes, specific dynamic action (SDA) and muscle contractions, exemplify this approach, offering insights into critical aspects of metabolism and muscle physiology. Investigations into muscle catch contractions observed in mussels, which maintain shell closure over long durations with minimal energy expenditure, offer valuable insights into regulation of muscle function and the interplay between neurotransmitters like dopamine and serotonin and muscle dynamics. Similarly, specific dynamic action, characterized by an increase in metabolic rate following food consumption, sheds light onto the energy utilization processes that is universal in a wide range of organisms. Through the lens of aquatic model organisms, specifically mussels and zebrafish, the mechanisms underlying SDA and muscle contractions can be better understood, providing valuable insights with broad implications for fields ranging from ecology to human health.

Catch Contractions

Although humans require ATP when contracting muscles, organisms like mussels can use catch contractions to hold their shells closed with little to no energy for extended periods (Funabara et al., 2007). Evolutionarily, this is beneficial because it offers long-term protection without significant energy expenditure. When vertebrate muscle cells experience depolarization, Ca^{2+} ions are released into the cell to initiate a cross-bridge cycle. Ca^{2+} binds to troponin, which unblocks myosin binding sites on actin filaments. Once myosin heads are tightly bound to actin, it uses energy from the release of P_i to drive the power stroke. This causes contractions while Ca^{2+} ions are still present in the cell (Malik et al., 2011). However, invertebrate muscles in catch condition can stay contracted in the absence of Ca^{2+}. Catch contractions are modulated by the conformation of the twitchin protein - when dephosphorylated, twitchin binds to actin and myosin to maintain the contracted state; when phosphorylated, it releases myosin from actin and relaxes the muscle (Figure 1A) (Funabara et al., 2007).

When serotonin (5HT) is applied to the mussel muscle cell, it binds to a G-protein coupled receptor that is linked to adenylate cyclase. Adenylate cyclase converts ATP to cAMP, which activates protein kinase A (PKA). PKA phosphorylates twitchin, and the change in twitchin conformation allows myosin to detach from the actin filaments and relaxes the catch contraction (Figure 1A). Adenylate cyclase, an enzyme involved in the phosphorylation process, is sensitive to 5HT and dopamine (DA) (Deterre et al., 1986). Given that 5HT acts as an activator that leads to the phosphorylation of twitchin, DA was hypothesized to similarly relax catch contractions and a combination of 5HT and DA would increase relaxation response.

METHODS

To test this hypothesis, the experiment was set up as indicated in Patek in terms of calibration curve, dissection and force transducer setup (2016). For each trial, a new mussel was induced to catch condition with 5-8 drops of 10^{-3}M acetylcholine (Patek, 2016). Once the muscle is at peak force, 5-8 drops of instant ocean (n=1), 10^{-3}M 5HT (n=3), 10^{-3}M DA (n=3), or a mixture of 10^{-3}M DA and 10^{-3}M 5HT (n=4) were added. Since the muscle did not relax after instant ocean was added in the control trial, the experiment with 5HT was terminated. Relaxation response was measured by how much the muscle relaxes from catch contraction due to the addition of chemicals (labeled as reduction from peak force) as well as the time it takes for this relaxation to occur (labeled as relaxation rate).

RESULTS

Catch contractions were induced in mussels using acetylcholine. After ocean water was added to the mussel in catch condition (as a control), there was no relaxation response. The control mussel showed no reduction from peak force, and thus a relaxation rate of 0N/s. The mussels treated with DA had a mean reduction from peak force that was less than those treated with 5HT, but were greater than control (Figure 1B). Similarly, the mean relaxation rate of DA-treated mussels was slower than that of 5HT-treated mussels (Figure 1C). There was no overlap between relaxation response data of 5HT-treated mussels and DA-treated

mussels – the minimum reduction from peak force of 5HT-treated mussels was greater than the maximum of DA-treated mussels (Figure 1B). The same can be said about the relaxation rate of mussels that were treated with DA versus 5HT (Figure 1C). This shows that DA had a similar, but lesser, effect on relaxation response than 5HT, supporting the hypothesis that DA would also initiate the phosphorylation cascade.

When a 5HT and DA mixture was applied, the reduction from peak force was in between that of 5HT and DA (Figure 1B). Correspondingly, the relaxation rate of mussels treated with 5HT+DA was also in between that of mussels treated with 5HT and DA (Figure 1C). The maximum value for reduction from peak force of 5HT+DA-treated mussels was within the range of 5HT-treated mussel data, while the minimum value of 5HT+DA-treated mussels was within the range of DA-treated mussel data (Figure 1B). Again, data on relaxation rate of 5HT+DA-treated mussels shows the same trend in overlap with 5HT-treated mussels and DA-treated mussels. This does not support the second part of the hypothesis, but could be explained by competition of 5HT and DA in the adenylate cyclase reaction.

DISCUSSION

DA was anticipated to relax catch contractions, as 5HT does. A combination of 5HT and DA was also expected to cause greater amplitude of relaxation from peak contraction and faster relaxation rate than 5HT alone. To assess this hypothesis, instant ocean, 5HT, DA, or a mixture of DA and 5HT were added to mussels in catch condition. The mussels treated with DA had a lower mean reduction from peak force and slower mean relaxation rate than those treated with 5HT, which supports the first part of the hypothesis. When a 5HT and DA mixture was applied, the reduction from peak force and relaxation rate were greater than those of mussels treated with DA, but less than those of mussels treated with 5HT, which differs from the second part of the hypothesis.

5HT is known to relax catch contractions by initiating a metabotropic cascade that results in twitchin phosphorylation – activate adenylate cyclase to generate cAMP, which triggers PKA to phosphorylate twitchin (Figure 1A) (Funabara et al., 2007). When twitchin is phosphorylated, the muscle becomes relaxed. Adenylate cyclase is coupled to both 5HT and DA receptors, which suggests that DA would have a similar effect as 5HT in catch relaxation (Gies, 1986). However, previous literature determined that DA has a lesser effect on muscle relaxation than 5HT, possibly because DA is a weaker activator of adenylate cyclase (Gies, 1986). At various concentrations of 5HT and DA, catch relaxation caused by 5HT occurs faster than catch relaxation caused by DA (Gies, 1986). Additionally, when DA concentration is incremented by an order of magnitude from 10^{-8}M to 10^{-4}M, relaxation response also increases as concentration increases; this indicates some dose-dependency in catch relaxation due to DA (Gies, 1986).

The method for this experiment was based on previous protocols, which found that the most effective and consistent dose of 5HT was 10^{-3}M, so the same dose of DA was used. As mentioned previously, research indicates that the effects of DA are dose-dependent (Gies, 1986). Addition of DA shows less reduction from peak force and slower relaxation rate than 5HT, and it is possible that a greater concentration of DA would produce a stronger response that is more like that of 5HT (Figure 1B). Similarly, greater concentration of DA in the combination of DA and 5HT would generate data with smaller value of standard deviation, since higher concentration of DA might increase relaxation response to be comparable that of 5HT. Testing a range of dosages and concentrations of DA would offer better insight into the mechanism of how DA relaxes catch contractions.

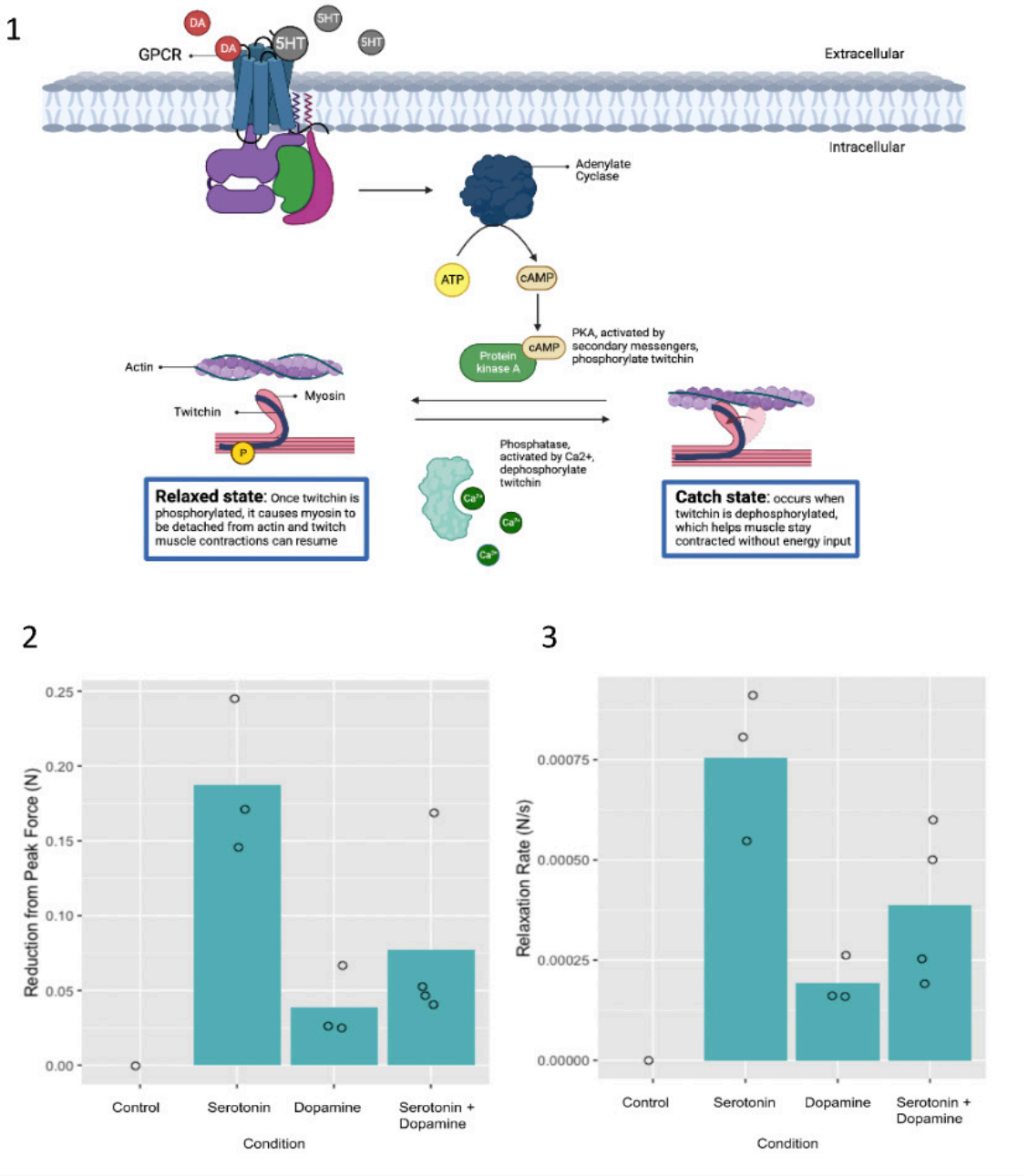

Figure 2: Relaxation response is first measured by the reduction from peak force (N). Column height represents mean and the dots represent the raw data points. When comparing mean reduction from peak force, 5HT-treated muscles (n=3) in catch condition had the greatest reduction from peak force and control (n=1) had the least. Dopamine-treated muscles (n=3) had a low reduction from peak force, but the response was still present. Muscles treated with a mixture of 5HT and dopamine exhibited an intermediate force reduction (between that of 5HT and dopamine).

Figure 3: Relaxation response can also be measured by rate, which was calculated by dividing the reduction from peak force (N) by time it took to relax (secs). Like Figure 1B, column height represents mean and the dots represent the raw data points. Both reduction from peak force and relaxation rate data show similar trends. 5HT-treated muscles in catch condition (n=3) had the fastest mean relaxation rate. Dopamine-treated muscles had a slower mean relaxation rate (n=3), but still greater than the control (n=1). Mussel muscles treated with both 5HT and dopamine have a mean relaxation rate between that of serotonin and dopamine (n=4).

Another limitation for the experiment is that only the amplitude of contraction (after catch is induced) and the change after 5HT and/or DA is added to the mussel were recorded, as well as the time it takes for this relaxation to occur. This yields data in the form of reduction from peak force and relaxation rate. However, it would be beneficial to measure the amplitude of contraction force in order to determine percentage of relaxation due to 5HT and/or DA. A small reduction from peak force could mean that the compound had little effect on catch relaxation, but it could also mean that initial catch contraction achieved a relatively low peak force. This change would not impact the data itself, but it would clarify the discussion of what the results mean.

Furthermore, DA agonist SKF3893 at 10^{-5}M is sufficient to relax catch contractions but does not increase the amount of cAMP in the muscle (Murakami et al., 1986). This presents the possibility that DA has an additional mechanism of hyperpolarization as well as adenylate cyclase activation. Typically, to initiate an action potential, presence of acetylcholine causes sodium channels to open and depolarize the muscle cell. If DA causes hyperpolarization, the cell would require greater influx of Na^+ ions to reach the threshold for action potential. This mechanism could be tested by comparing the concentration of acetylcholine needed to cause catch contraction with and without DA added beforehand. Further research in this direction would elucidate the role of DA in catch contraction as well as catch relaxation in mussels.

Specific Dynamic Action

Metabolic rate is influenced by digestion through a phenomenon called specific dynamic action (SDA), in which metabolic rate increases after food consumption (Rubner, 1902). Metabolic rate is a fundamental physiological parameter that can have significant implications on an animal's survival and fitness outcomes, prompting widespread inquiry of the SDA effect (Burton et al., 2011). Previous research has established the presence of SDA across a wide range of organisms, suggesting it is a common physiological phenomenon (Jobling & Davies, 1978). However, there are still gaps in understanding regarding the mechanisms and magnitude of SDA across different species and contexts. For example, recent studies have suggested that the magnitude of SDA in fish may increase in duration but decrease in magnitude as temperature decreases (Tirsgaad, 2015). Understanding these factors and their effects on SDA can provide insights into the ecological and evolutionary significance of this phenomenon, as well as inform practical applications such as animal feeding and husbandry practices in aquaculture and agriculture.

To study the scope of SDA, we designed an experiment to compare the metabolic rate of fed and fasted zebrafish populations We hypothesized that 1) metabolic rate would be higher in the exercise condition due to additional muscle energy expenditures and 2) metabolic rate would be higher in the fed condition due to the SDA effect. By testing our hypotheses about the effects of exercise and feeding on metabolic rate, we aimed to contribute to our understanding of the underlying mechanisms of SDA and its potential implications in energy expenditures.

Understanding the impact of these factors on metabolic rate is crucial for predicting energy expenditures and ultimately, survival and fitness outcomes.

METHODS

To study the scope of specific dynamic action (SDA), an experiment was designed to compare the metabolic rate of fed and fasted zebrafish populations. The experiment was conducted using a single previously fasting zebrafish, which was acclimated to the flask's environment for 15 minutes before the start of the experiment. Following the acclimation period, the zebrafish was either fed 10mg of fish food (Fig. 1) or fasted for 30 minutes. The use of a standardized fish food ensured that the nutritional content of the food was consistent between individuals and that any observed differences in the SDA response could be attributed to other factors, such as activity level or the fed versus fasted state.

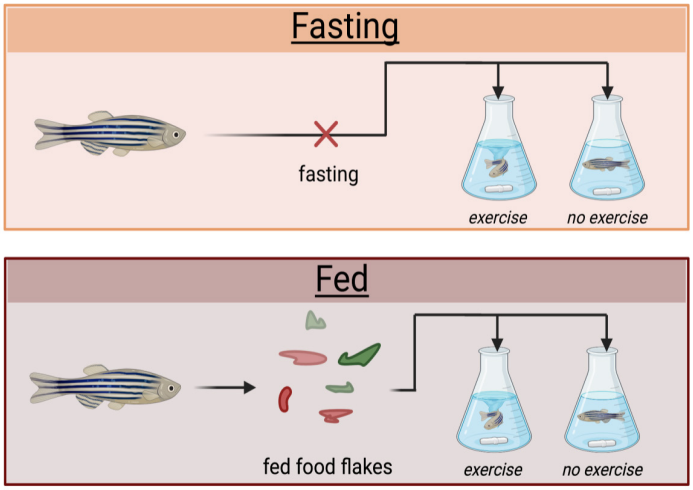

Figure 4. The experimental design, adapted from the design by Trueblood, featured both a fasted (orange) and fed (dark red) population (2015). The populations were then subjected to either an exercise or no exercise condition. In the exercise condition, the zebrafish were active due to the creation of a current by a magnetic stir bar. In the no exercise condition, the zebrafish were not subjected to any external stimuli.

After the feeding or fasting period, the metabolic rate of the zebrafish was measured using an oxygen probe for 15-30 minutes, as described by Trueblood (2015). For both populations, a condition of exercise and no exercise was implemented to measure the effect of physical activity on metabolic rate and SDA. In the exercise condition, the zebrafish were subjected to physical activity by creating a current in the flask using a magnetic stir bar. The no exercise condition, on the other hand, involved no external stimuli, allowing the zebrafish to remain at rest. To control for the possible magnetic field influence of the stir plate, all flask environments were placed on top of the magnetic stir plate with a stir rod inside, even if it was inactive. In all trials, the casper strain of zebrafish was used to control for genetic variation. All zebrafish were held in the same tank environment prior to the initiation of the experiment and fasted for 12 hours to reduce discrepancies.

RESULTS

A total of eleven trials were conducted. In both the no exercise and exercise condition (Fig. 2), the fasted fish exhibited a higher mean mass-specific metabolic rate (MSMR). Across all conditions, the no exercise while fasting condition exhibited the highest mean MSMR, 0.0011 mL O2/ s*g. In contrast, the exercise while fed condition exhibited the lowest mean MSMR, 0.00022 mL O2/ s*g. The other two conditions, no exercise while fed and exercise while fasting, had respective mean MSMRs of 0.0070 and 0.0071 mL O2/ s*g. Unexpectedly, the exercise groups did not exhibit a higher mean MSMR in comparison to the no exercise groups. The range of values across all groups was 0.002 to 0.0014 mL O2/ s*g, suggesting a large variance between the data points.

In terms of distribution, the fasted exercise fish had the widest range of data values, 0.00022 to 0.0014 mL O2/ s*g. In contrast, the fed exercise fish exhibited the tightest distribution and range, 0.00011 to 0.00027 mL O2/ s*g. It is important to note, however, that the exercise while fasting fish had a significant outlier at 0.0014 mL O2/ s*g. It was significantly different from the rest of the group, whose data points otherwise ranged from 0.002 to 0.006 mL O2/ s*g. This likely contributed to the group's wide distribution and larger mean (Fig. 1B). This data point was also the maximum MSMR measured across all groups.

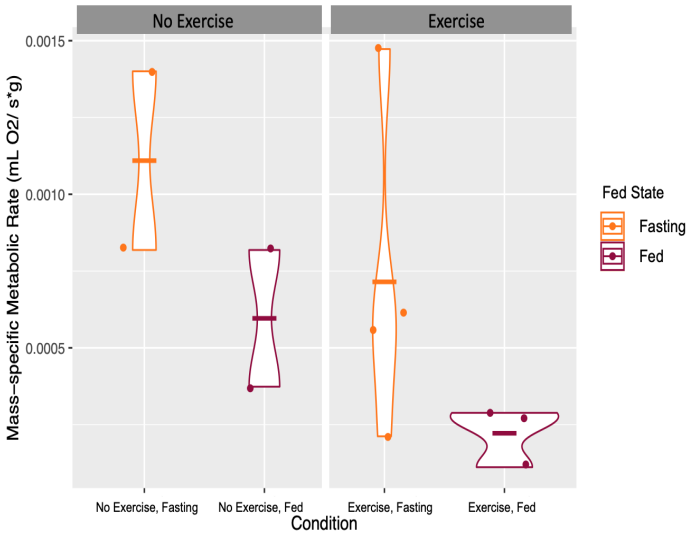

Figure 5. Specific dynamic action (SDA) was not observed in this experimental design, as fasting fish exhibited a higher mass-specific metabolic rate (mL O2/ s*g). The fasted fish exhibited a higher mean mass-specific metabolic rate (mL O2/ s*g), as illustrated by the horizontal line, in both the no exercise and exercise condition. In terms of distribution, the fasted exercise

fish had the widest distribution of data values, as shown by the long length of the violin plot. In contrast, the fed exercise fish exhibited the tightest distribution.

DISCUSSION

We hypothesized that the metabolic rate would be higher in the fed condition due to the SDA effect. However, the fasted fish exhibited a higher mean MSMR (Fig. 1B) in both exercise conditions. Current literature suggests that metabolic rate should increase due to the additional energy expenditures of digestion associated with SDA (Brown & Cameron, 1991). Similar investigation in plaice fish found an increase of metabolic rate, ranging from 141.8% to 191.6%, in the following 2 days after feeding (Jobling & Davies, 1978). However, the results in zebrafish contradict previous findings. The fed zebrafish population exhibited a lower mean MSMR in both exercise conditions, challenging our proposed hypothesis. Because fed fish did not exhibit a higher metabolic rate as expected, these results suggest that the SDA effect did not occur.

Analytically, the absence of SDA is unexpected. Physiologically, the energetic costs associated with the absorptive processes of digestion should result in a noticeable in increase in metabolic rate (Hailey, 1987). Meal heating, enzyme secretion, protein catabolism, and intestinal absorption have been recorded to contribute to SDA metabolic surges, as demonstrated in pit viper experimentation (Tsai et al., 2008). Similar digestive processes occur in zebrafish digestion, drawing doubt to the lack of observed metabolic rate increase. However, a few explanations may justify the absence of SDA. Firstly, it is possible that SDA occurs after the 15-30 min. window measured, due to the latency of digestion. Previous investigation with goats has recorded a delay in which digestive processes take place, possibly due to the necessity for enzyme production and associative protein scaling (Magee, 1924). As such, SDA may still have occurred in the zebrafish populations, but beyond the 15-30 min. window. Future experimentation should include a longer measurement window to ameliorate this limitation.

Moreover, acute stress from a new environment may have limited digestive processes and thereby minimized the SDA effect. Previous investigation has noted that the transport and variance in holding containers associated with laboratory conditions can increase zebrafish cortisol (Dhansari et al., 2013). Increased cortisol is known to stimulate fat and carbohydrate metabolism to increase glucose availability, as shown in prior human injection (Brillon et al., 1995). Thus, acute transfer stress could have increased metabolic rate across all groups, potentially reducing the magnitude of SDA's effect. To ameliorate this phenomenon, a greater acclimation period should be implemented to reduce acute stress, which may prevent energy allocation to digestive processes and thus reduce the magnitude of SDA.

Beyond the short measurement window and acclimation period, the experimental design may have been further limited. Meals with higher protein compositions have been recorded to have higher SDA effects in plaice fish, likely due to additional protein catabolism (Jobling & Davies, 1978). As such, the fish food composition may have been lacking in protein, which would limit the magnitude of SDA. To fix this, future experiments should use high-protein compositions to increase SDA presence and magnitude. Additionally, the use of Parafilm to cover the flask may have led to an incomplete seal, allowing gaseous oxygen to diffuse into the water. The diffusion of oxygen would increase oxygen readings, lowering the measured metabolic rate and thereby disguising any SDA effect. The wide distribution of data values for each condition, as shown through the long lengths of the violin plots (Fig. 1B), suggests that the readings were not consistent, and this insufficient seal may have occurred. Future experiments should use a modified silicone stopper, which would create a better seal and tighter distribution of data values.

Furthermore, additional inquiry should investigate the potential variance of SDA across different life stages. Metabolism is known to fluctuate across an individual's lifespan, notably declining as humans age (Milward et al., 1997). Intestinal efficiency also declines with age due to changes in gut microbiota and other factors (Coudray et al, 2006). As such, future experimentation should investigate if increased age, along with declines in intestinal efficiency, reduces the SDA response. Overall, while the results did not

record an SDA effect, the data suggests SDA may be influenced by more environmental conditions that previously believed.

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FIGURES

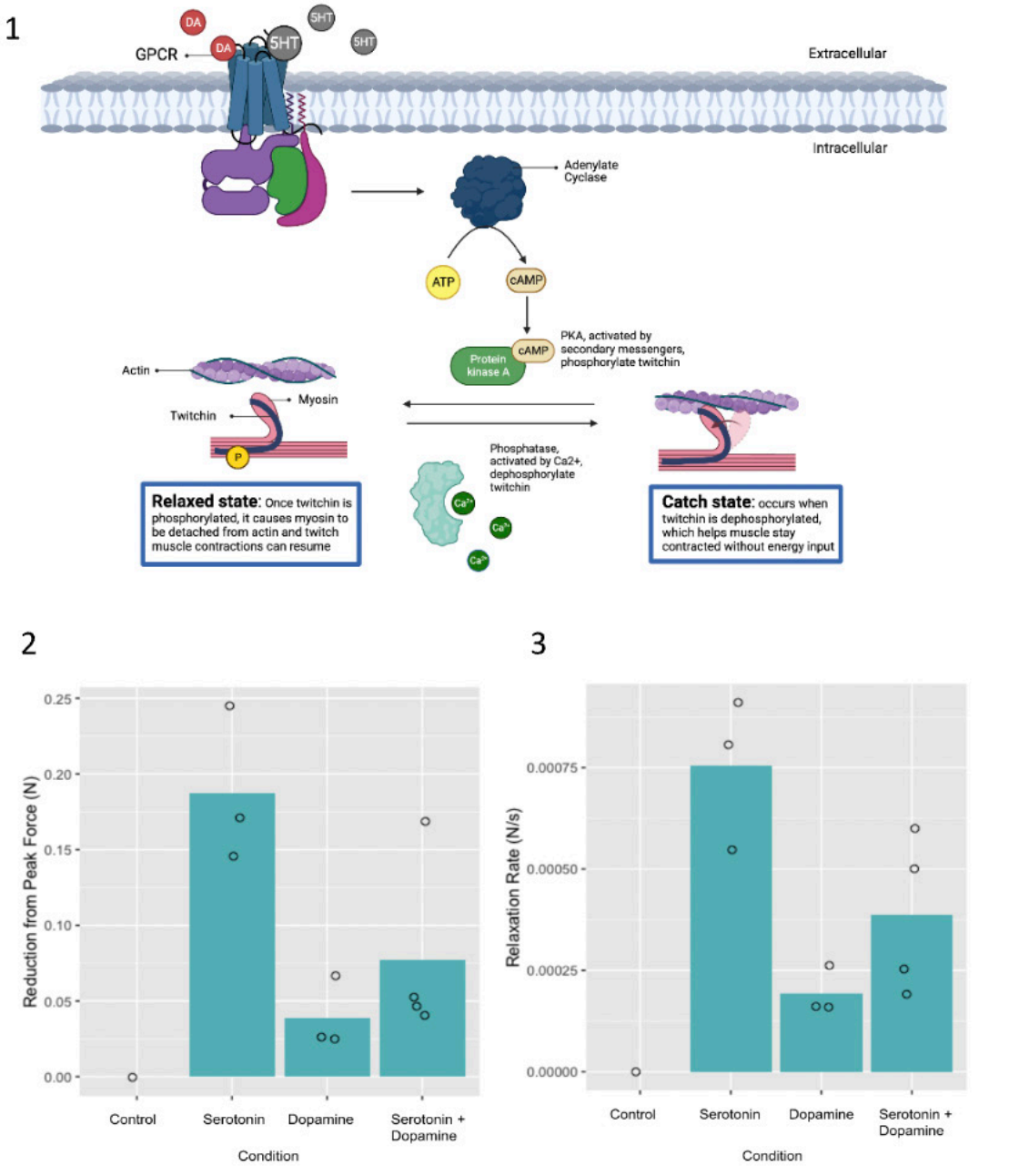

Figure 1: Dopamine and serotonin both activate the signaling cascade to phosphorylate twitchin and relax catch contractions in mussels – 5HT's effect on relaxation response is stronger than DA's, and relaxation response to a combination of 5HT and DA is greater than that of DA alone, but less than that of 5HT alone.

A) Serotonin, and dopamine (not pictured), are extracellular signaling molecules that bind to G-protein coupled receptors (GPCR). GPCRs set off an intracellular signaling cascade that activates adenylate cyclase, an enzyme that converts ATP to cAMP. cAMP is a secondary messenger that activates protein kinase A (PKA), which phosphorylates twitchin. Once phosphorylated, twitchin releases actin and myosin and relaxes the catch contraction. To begin a catch contraction, a phosphatase activated by Ca^{2+} dephosphorylates twitchin, which allows twitchin to bind myosin and actin. Adapted from Funabara et al.,

2007.

B) Relaxation response is first measured by the reduction from peak force (N). Column height represents mean and the dots represent the raw data points. When comparing mean reduction from peak force, 5HT-treated muscles (n=3) in catch condition had the greatest reduction from peak force and control (n=1) had the least. Dopamine-treated muscles (n=3) had a low reduction from peak force, but the response was still present. Muscles treated with a mixture of 5HT and dopamine exhibited an intermediate force reduction (between that of 5HT and dopamine).

C) Relaxation response can also be measured by rate, which was calculated by dividing the reduction from peak force (N) by time it took to relax (secs). Like Figure 1B, column height represents mean and the dots represent the raw data points. Both reduction from peak force and relaxation rate data show similar trends. 5HT-treated muscles in catch condition (n=3) had the fastest mean relaxation rate. Dopamine-treated muscles had a slower mean relaxation rate (n=3), but still greater than the control (n=1). Mussel muscles treated with both 5HT and dopamine have a mean relaxation rate between that of serotonin and dopamine (n=4).

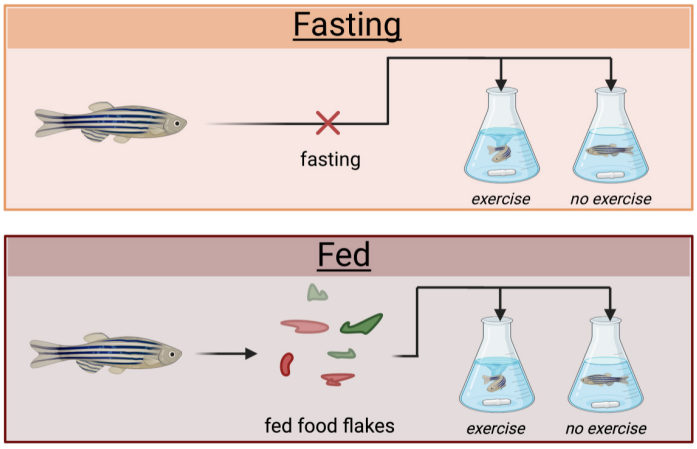

Figure 4. The experimental design, adapted from the design by Trueblood, featured both a fasted (orange) and fed (dark red) population (2015). The populations were then subjected to either an exercise or no exercise condition. In the exercise condition, the zebrafish were active due to the creation of a current by a magnetic stir bar. In the no exercise condition, the zebrafish were not subjected to any external stimuli.

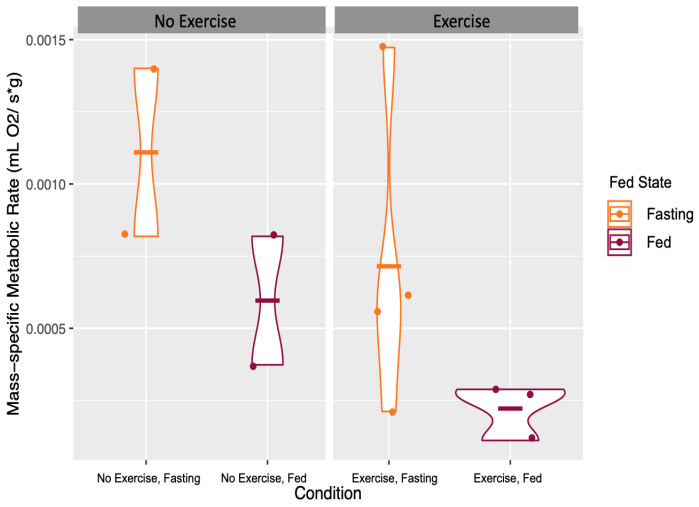

Figure 5. Specific dynamic action (SDA) was not observed in this experimental design, as fasting fish exhibited a higher mass-specific metabolic rate (mL O2/ s*g). The fasted fish exhibited a higher mean mass-specific metabolic rate (mL O2/ s*g), as illustrated by the horizontal line, in both the no exercise and exercise condition. In terms of distribution, the fasted exercise fish had the widest distribution of data values, as shown by the long length of the violin plot. In contrast, the fed exercise fish exhibited the tightest distribution.

A Correlational Study of the Relationship Between ChatGPT Usage & Cognitive Flexibility

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Article Synopsis

As artificial intelligence tools like ChatGPT become increasingly prevalent in educational settings, it is crucial to understand how their usage influences students' cognitive processes. Szymkiewicz et al. investigate the relationship between the frequency of ChatGPT usage and cognitive flexibility among university students. They find that frequent users (those who used ChatGPT for more than 50% of their academic work) exhibit greater cognitive flexibility compared to infrequent users, as evidenced by less of a decrease in response accuracy when switching between two tasks. While further research is needed to fully understand the implications of these results, they may help to destigmatize the use of AI tools in educational contexts.

Graphic by Amanda Li

A Correlational Study of the Relationship Between ChatGPT Usage & Cognitive Flexibility

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Abstract

Amidst the growing integration of AI into educational practices, concerns arise regarding how unregulated interaction with AI outside the structured classroom environment may influence university students' cognitive functions. This research examines the differential effects of ChatGPT usage intensity on university students' cognitive flexibility. The researchers hypothesize a negative correlation between ChatGPT usage and cognitive flexibility, measurable by increased response times and decreased accuracy between the two tasks of the Arrow Switch Test. We employed this test to contrast the performance of frequent (participants who used ChatGPT $\geq$ 50% of the time on academic assignments) and infrequent (participants who used ChatGPT $\leq$ 35% of the time on academic assignments) groups. For the purpose of this study, infrequent users were used as a control group. Both groups displayed a statistically significant decrease in task accuracy—highlighting the Arrow Switch Task's efficacy in assessing cognitive flexibility—and response time between the first and second task. Notably, infrequent users of ChatGPT demonstrated a larger decline in accuracy and response time following changed task conditions compared to frequent users. This finding calls for further investigation into the longitudinal effects of AI tools on learning processes, necessitating a larger sample size and a more granular analysis of usage patterns to understand the subtleties of AI's impact on cognitive flexibility.

Keywords: artificial intelligence, education, cognitive flexibility, correlation, ChatGPT

INTRODUCTION

With swift advancements in technology, Artificial Intelligence (AI) tools like ChatGPT have become commonplace in educational settings. The prospective usage of AI inside classrooms has been promising with preliminary research showing that educational AI could improve the quality of education college students receive (Alam, 2022). AI has also proven to be useful to personalize education through resources like media recommendations, as it increases students' engagement and abilities (Huang et al., 2023).

Furthermore, AI has been shown to increase academic performance, self-efficacy, and motivation in students when used as a tool to provide real-time feedback during the completion of supplemental course review sheets (Lee et al., 2022). Research on AI as a classroom tool has only begun, and there is a gap exploring how AI not regulated by educators affects student performance. Although AI is anticipated to be a beneficial tool for educators and students within controlled classroom settings, the focus of this paper is on the potential negative consequences of students' unrestricted use of AI for academic purposes outside classroom settings (Timms, 2016).

Specifically, unrestricted ChatGPT usage could lead students to complete academic tasks with less cognitive involvement. ChatGPT has been characterized by scholars as high-tech plagiarism leading to learning avoidance (Chomsky et al. 2023). As such, the researchers set out to explore how unrestricted usage of ChatGPT on academic tasks influences students' cognitive flexibility, a critical aspect of learning and academic performance. Cognitive flexibility—the ability to adapt thinking and approach to varying tasks—is a significant predictor of academic success (Kercood et al., 2017). This adaptability encompasses not only the ability to switch between tasks or topics with ease but also involves the aptitude for understanding and applying concepts across different contexts. For instance, it enables students to leverage mathematical formulas learned in one scenario to solve science problems in another, or to draw on historical events to deepen their analysis of literature. This research will examine whether and to what extent ChatGPT usage correlates with this crucial intellectual capability.

We hypothesize that frequent use of ChatGPT for academic purposes decreases cognitive flexibility, observable through increased response time and decreased accuracy between the two tasks of the Arrow Switch Test. The Arrow Switch Test was implemented in order to quantify cognitive flexibility. This test required participants to look at an arrangement of arrows, and, based on the color of the arrows, respond accordingly. One color would require the participants to respond with the direction of the rightmost arrow in the arrangement, while the other color would require the participants to respond with the direction of the leftmost arrow. Halfway through the test, participants were prompted to switch how they responded so that the color cues were flipped. The portions of the test before and after the switch were labeled as Task 1 and Task 2, respectively. Both accuracy and response times were recorded. By investigating this relationship, the study seeks to contribute to the broader discourse on the role AI plays in influencing cognitive processes.

METHODS

Participants

The study sample comprised 22 American undergraduate students studying at the Danish Institute for Study Abroad, selected primarily due to accessibility and their willingness to participate voluntarily. There was no intentional imbalance in gender participation: 10 females participated, and 12 males participated.

The participants were divided into two groups based on their self-reported usage of ChatGPT for academic purposes: the control group (14 students) reported using ChatGPT less than 35% of the time, while the experimental group (8 students) used ChatGPT more than 50% of the time. Students were asked to measure their usage of ChatGPT in relation to all of their classes dating back to ChatGPT's launch on November 30, 2022. The division was intended to contrast the cognitive flexibility between frequent and infrequent users of ChatGPT. Both females and males were present in both the control and experimental groups.

Inclusion criteria required that participants had access to AI tools in their academic environment and that they spoke English fluently. Students with diagnosed cognitive impairments affecting task performance were excluded to maintain result integrity.

Materials

An initial survey was conducted through Google Forms to quantify each student's usage frequency of ChatGPT for academic tasks. Participants were asked what percentage of their academic work they complete using ChatGPT, and when they do use it, what percentage of the time they integrate ChatGPT generated text into their work. "Use" was defined as prompting ChatGPT for any type of response without regard for whether this response would be directly integrated (i.e. copy and pasted) into their academic work or not. Integration of content was defined as copying and pasting unaltered content and incorporating altered content (paraphrasing, restructuring, rewording, reworking, adapting, or rewriting) into personal work as opposed to using ChatGPT for solidifying one's understanding of concepts.

A computerized Arrow Switch Test was developed to quantify cognitive flexibility. This task was designed to record response accuracy and time, providing a measure of the participant's ability to shift cognitive strategies. The task was run in a controlled laboratory setting on desktop computers equipped with E-Prime software to ensure precise timing and data collection. Participants interacted with the task using standard keyboards using the hand of their preference.

Arrow Switch Test

The Arrow Switch Test was modeled after the principles of the Wisconsin Card Sort Test (Grant & Berg, 1948). The Arrow Switch Test required participants to focus on a series of five arrows of the same color, either purple or orange, displayed on a computer screen. Participants were tasked with responding to the arrows' orientation by pressing designated keys on a keyboard. The responses were tied to the colors of the arrows, requiring participants to employ attention to detail and color-orientation association (i.e., if the arrows were orange, the participants pressed the arrow key corresponding to the direction of the rightmost arrow, and if the arrows were purple, the leftmost arrow of the five).

The first task, Task 1, of the test consisted of 42 trials, after which the investigation entered its critical phase: the switch. At this juncture, the previously learned color-response associations were reversed without prior notice to the participants. They were explicitly informed of the new associations and thereafter completed 42 more trials under these new conditions (Task 2). This sudden reversal in task requirements was designed to measure cognitive flexibility, challenging the participants' ability to adapt to new rules and to modify their cognitive strategies accordingly. Therefore, Task 1 and Task 2 together comprise the singular Arrow Switch Task that participants undertake.

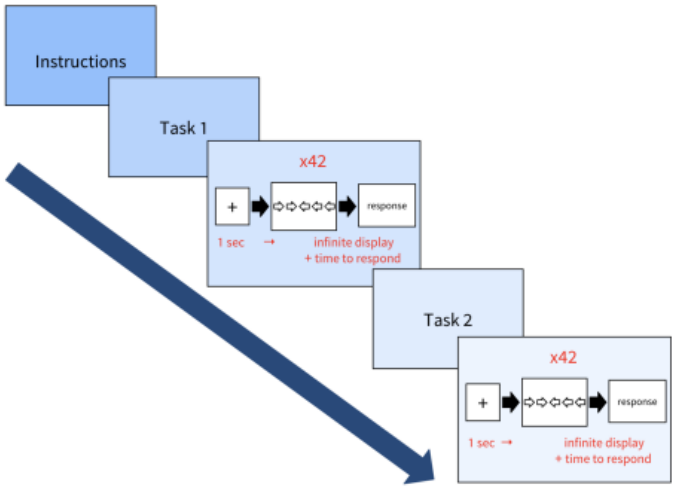
Procedure

Before participation, students completed an informed consent process outlining the study's objectives, anonymous and confidential treatment of participant data, and the right to withdraw at any time without penalty. Participants then completed the online survey quantifying their frequency of ChatGPT usage on academic tasks. Following the survey, participants were brought into the laboratory within the same week and introduced to the Arrow Switch Test. The entire task sequence was conducted in a quiet and controlled laboratory environment. Participants completed the test individually in a temperature-controlled environment with a singular desk, chair, and computer. This environment was maintained to ensure the accuracy of response data and to minimize external variables affecting the participants' performance.

Throughout the task, response times and accuracy were recorded for each participant through E Prime software. These data were central to the study's analysis, providing objective measures of the impact of ChatGPT usage on cognitive flexibility.

Figure 1

Arrow Switch Test



Analyses

The initial analysis involved conducting both Wilcoxon Tests and t-Tests to determine the significance of the data. However, it became apparent that the small sample size precluded the assumption of normality, leading to non-normal distribution of the data. Consequently, the skewness of the data varied, being either left or right, depending on the specific variable and group under examination. A Wilcoxon Test tests for differences between group means of independent samples when the data is not normally distributed. Therefore, due to its accurate reflection of the data, only the Wilcoxon Test was used for analysis. Accuracies of individuals were averaged across the 42 trials in the two tasks separately. Response times of correct answers only were analyzed.

RESULTS

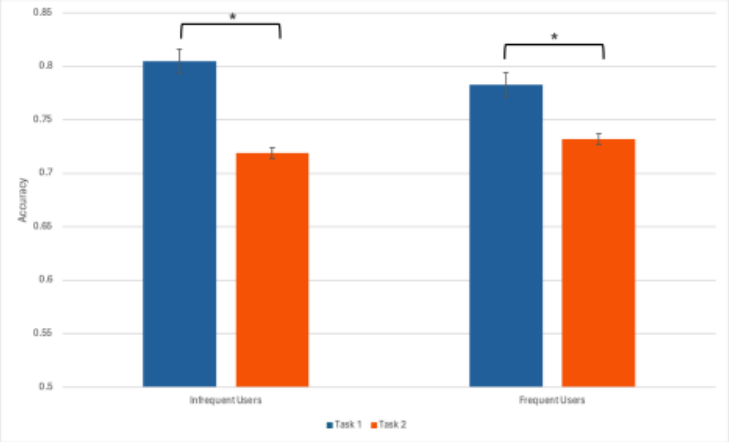
The evaluation of cognitive flexibility through the Arrow Switch Test revealed significant decreases in performance in terms of accuracy and response times across tasks and user groups.

Accuracy Analysis

Comparing the accuracy across all participants between Task 1 and Task 2 revealed a significant decrease from an average accuracy of 79.7% in Task 1 to 72.4% in Task 2. In this case the t-score = 22.0 and p = 0.0001 which meant that the mean accuracy from Task 1 was statistically significantly different from the mean accuracy of Task 2, illustrating that the Arrow Switch Test effectively measured cognitive flexibility by initially acclimating participants to Task 1 before requiring them to change their responses in Task 2. This expected drop in accuracy underscores the test's ability to challenge cognitive adaptability. Upon segmenting participants into infrequent and frequent users, the decline in accuracy between Task 1 and Task 2 remained significant for both groups, as illustrated in Figure 2. To compare across groups, eight infrequent users were randomly selected and compared with eight frequent users, revealing that the average accuracy drop from Task 1 to Task 2 was -0.086 for infrequent users and -0.051 for frequent users, indicating a greater decline in accuracy among infrequent users (see Figure 3). This difference in accuracy is calculated by subtracting Task 1 scores from Task 2 scores. The Wilcoxon Test results for the comparison between the difference of accuracy between Tasks in frequent users versus infrequent users yielded p = 0.0 and t-score = 4.0. Yet, considering each task separately, no statistical significance was found between the accuracies of infrequent and frequent users, suggesting that the overall usage was not correlated with different levels of performance in individual tasks.

Figure 2

Accuracies in the Arrow Switch Test Separated by Usage

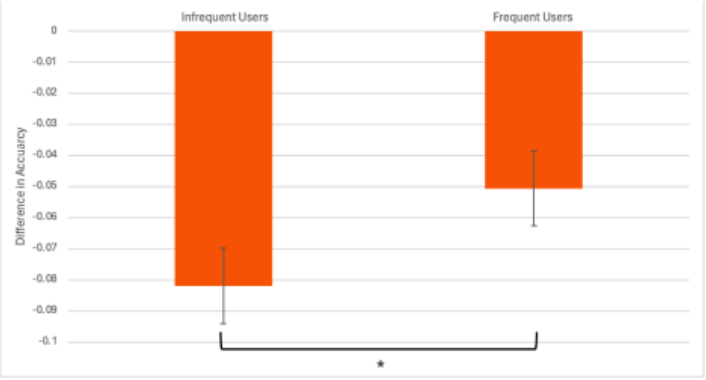


Note. The average accuracy for infrequent users on Task 1 was 80.5% and on Task 2 was 71.9%. This difference was significant ($p < 0.01$ and t-score = 1.0) according to the Wilcoxon Test results. The average accuracy for frequent users on Task 1 was 78.3% and on Task 2 was 73.2%. This difference was significant ($p < 0.01$ and t-score = 8.0) according to the Wilcoxon Test results. The same analyses for accuracy were done with ChatGPT integration, however no statistically significant results were found.

* indicates a $p < 0.01$

Figure 3

Differences in Accuracies on the Arrow Switch Test Separated by Usage



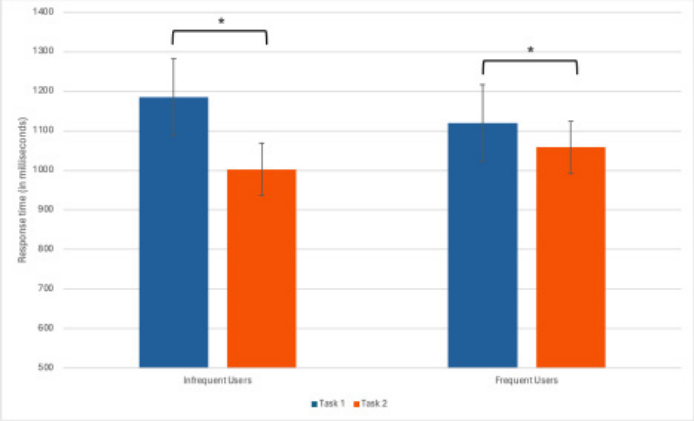
Note. * indicates $p < 0.01$

Response Time Analysis

When comparing the response time of all users in Task 1 and Task 2, there was a significant decrease in response time between the two tasks ($p < 0.01$ and t-score = 58.0) with the average response time in Task 1 being 1.163 seconds and the average response time in Task 2 being 1.022 seconds. The mean value of the response time for Task 1 across all users was statistically significantly different from the mean response time for Task 2. When participants were divided into infrequent and frequent users, the significant difference in response times between the two tasks persisted within both groups (refer to Figure 4). To compare across groups, eight infrequent users were randomly selected to compare the differences in response times in the two tasks between infrequent users and frequent users. The eight randomly selected infrequent users had an average difference in response time of -132.8 milliseconds between the two tasks while frequent users had an average difference in response time of -61.29 milliseconds (see Figure 5). Regarding the differences in response times between the two tasks, the Wilcoxon test results found that there was a significant difference between infrequent users and frequent users ($p < 0.01$ and t-score = 17.0). However, when comparing the response times of infrequent and frequent users within each task, there was no statistically significant difference between these two groups: their response times in Task 1 were not found to be significantly different from each other, and similarly, their response times in Task 2 were not significantly different from each other.

Figure 4

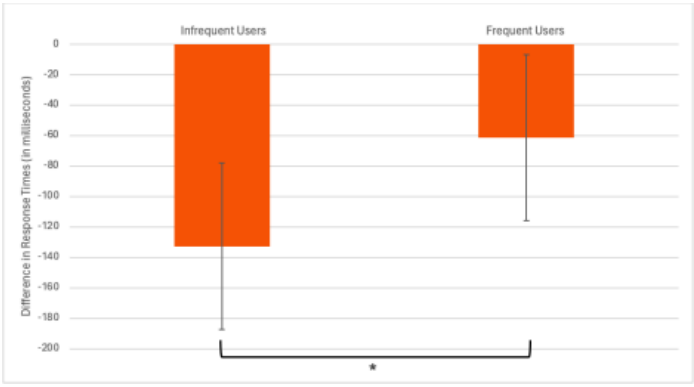
Response Times in the Arrow Switch Test Separated by Usage



Note. The average response time for infrequent users on Task 1 was 1.186 seconds and on Task 2 was 1.002 seconds. This difference was significant ($p < 0.01$ and t-score = 17.0) according to the Wilcoxon Test results. The average response time for frequent users on task 1 was 1.120 seconds and on task 2 was 1.059 seconds. This difference was also significant ($p < 0.01$ and t-score = 13.0) according to the Wilcoxon Test results. The same analyses for response time were done with ChatGPT integration, but no statistically significant results were found. * indicates $p < 0.01$

Figure 5

Differences in Response Times on the Arrow Switch Test Separated by Usage



Note. * indicates $p < 0.01$

DISCUSSION

The results do not support the hypothesis that there is a negative correlation between ChatGPT usage and cognitive flexibility as measured by increased response times and decreased accuracy on a cognitive flexibility task. The infrequent users demonstrated a larger average difference of both accuracy and response time between the two tasks compared to the frequent users. In addition, response times for all groups decreased between Task 1 and Task 2. This suggests participants may have felt more comfortable responding to Task 2 given its similarity to Task 1. There was no significant difference observed between frequent and infrequent users in each task, suggesting that both groups have comparable abilities in handling standard and switched tasks independently. However, the significant difference between the two groups' change in accuracy in the two tasks indicates that frequent users may possess greater cognitive flexibility compared to infrequent users, leading to less of a performance drop when the task rules are changed (Figure 3). This finding directly contradicts the original hypothesis, which anticipated that infrequent ChatGPT users would exhibit superior cognitive flexibility. Furthermore, no statistically significant data was found that correlated the amount of integration of ChatGPT generated responses with accuracy or response times between the two tasks.

One possible explanation for the observed enhanced cognitive flexibility among frequent ChatGPT users could be related to the concept of 'transfer of learning.' Transfer of learning refers to the application of knowledge and skills acquired in one context to a different context (Perkins & Salomon, 1992). Frequent interaction with the dynamic and variable environment of ChatGPT may have facilitated the development of transferable cognitive skills, such as adaptability and cognitive flexibility, which can be applied to novel tasks like the Arrow Switch Test (Barnett & Ceci, 2002). However, the correlation reported in this research cannot be assumed to be a direct connection and other possible variables could mediate this relationship.

Another explanation could be linked to the notion of 'desirable difficulties' in learning. Desirable difficulties refer to learning conditions that may initially impede performance but lead to long-term retention and transfer (Bjork & Bjork, 2011). The challenges encountered by frequent ChatGPT users

in navigating between AI-generated and human-generated content could act as desirable difficulties, promoting deeper processing and more flexible cognitive strategies (McDaniel & Butler, 2011).

Through the Arrow Switch Test, a decline in accuracy between tasks was observed across all participants, indicating a measurable impact on cognitive performance when transitioning between tasks. The observed decrease in accuracy is consistent with the established literature indicating that cognitive performance can be influenced by the introduction of new task conditions, particularly when these involve a switch in cognitive strategy (Cañas et al., 2006). The decrease in response time was unexpected but can be explained by the fact that both tasks were similar, and thus participants may have had a false sense of comfort and familiarity on the second task despite the rule switch.

It is important to note that the Arrow Switch Test does not require higher-level thinking or integration of information. It is possible that frequent ChatGPT users have advanced adaptability skills for routine tasks but would be at a disadvantage with tasks requiring higher level cognition. In academic contexts, frequent users may use ChatGPT to perform the higher level analysis that infrequent users would perform themselves, and thus infrequent users would be more accustomed to tasks requiring a higher level of cognition.

Interestingly, the Wilcoxon Test findings suggest that infrequent users of ChatGPT may approach cognitive tasks with a strategy that favors speed over accuracy. This group demonstrated a significant decrease in accuracy yet faster response times on the second task, indicating a potential over-reliance on initial task learning, which did not transfer well when the task conditions changed. One plausible explanation for this observation could be rooted in the cognitive processing styles of the infrequent ChatGPT users. These individuals might favor a heuristic approach to problem-solving, which relies on intuitive, rule-of-thumb strategies that are faster but less precise. This heuristic processing style is generally more efficient in terms of response time but can lead to errors when the task complexity increases or when there is a need to adapt to new rules or conditions (Hjeij & Vilks, 2023).

In contrast, frequent users exhibited a significantly smaller change in response time, implying a more measured approach to the task switch. The results suggest that these individuals may prioritize maintaining accuracy over increasing speed, which could reflect a different aspect of cognitive flexibility—namely, the ability to maintain performance stability in the face of changing task demands (Braem et al., 2018). It is also possible an interest in maximizing performance underlies both more frequent use of ChatGPT and more effort made on the task.

It is also important to note that while the convenience sampling employed in this study offers valuable insights, it also introduces potential limitations, including a selection bias and the inherent challenges of self-report measures. These limitations may affect the generalizability of the findings and the accuracy of reported behaviors and attitudes. Future research should aim to address these limitations by employing more diverse and representative sampling methods and by incorporating objective measures of ChatGPT usage.

The study also did not categorize participants based on their ChatGPT subscription levels, such as GPT Pro, which may provide more human-like interactions. This limitation suggests that future research could explore how different levels of AI sophistication, afforded by various subscription models, influence cognitive flexibility and user dependency.

In general, further research with a larger sample size will be necessary to assume normality and to solidify results. Potentially confounding variables should be tested for such as concrete intelligence measures (i.e. IQ). Future research should aim to explore the boundaries and specificities of this observed phenomenon. It would be useful to examine whether the increased adaptability skills of frequent ChatGPT users extend to more complex cognitive tasks, which require deeper analytical thinking and comprehension. To explore if the benefits observed in simpler tasks extend to more complex scenarios, future research could incorporate tasks demanding higher-order cognitive processes, like problem-solving and critical thinking. Examples include tasks that integrate cognitive flexibility with complex puzzles, testing participants' abilities in logical deduction and strategic planning, or applying cognitive flexibility in critical evaluation scenarios, requiring the analysis

of debate arguments to differentiate between strong and weak evidence. Additionally, given the relatively recent introduction of ChatGPT, longitudinal studies could be valuable to assess the impact of prolonged and sustained use on cognitive flexibility. The duration of ChatGPT usage should be investigated as a potential variable influencing the adaptability of cognitive strategies.

Furthermore, it is crucial to acknowledge the stigma associated with the use of ChatGPT in academic contexts. This stigma, often stemming from concerns about academic integrity and the appropriate use of AI in educational environments, could influence participants to underreport their engagement with ChatGPT. Implementing more precise and objective methods to quantify ChatGPT usage would enhance the reliability of research findings, but this approach presents potential challenges regarding privacy concerns. Future studies must therefore balance the need for accurate data collection with ethical considerations surrounding the privacy and autonomy of participants.

CONCLUSION

This study's exploration into the relationship between ChatGPT usage and cognitive flexibility has opened new avenues for understanding how interaction with AI may influence or may correlate with cognitive functions. Contrary to the initial hypothesis, the findings suggest that frequent users of ChatGPT may exhibit enhanced cognitive flexibility compared to infrequent users. In an era where AI is becoming increasingly embedded in our daily lives, the findings suggesting enhanced cognitive flexibility among frequent users of ChatGPT offer a counter-narrative to concerns about the potential cognitive drawbacks of frequent AI interaction. This research provides a nuanced perspective on how technology, often perceived as a crutch, might instead be fostering certain cognitive skills in users. The implication that engagement with AI could enhance adaptability and flexibility in cognitive tasks has profound implications for how we perceive, integrate, and utilize AI in various sectors, particularly in education and workforce development. This intriguing result not only challenges preconceived notions about the impact of AI interaction on cognitive abilities but also invites further investigation into the nuances of this relationship.

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Meet Our Editing Team

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Julia Davis,
President & Editor-in-Chief

Julia Davis (Trinity ‘24) is majoring in Neuroscience and pursuing a certificate in Science and Society from Boston, MA. She is also the Editor-in-Chief of Vertices’ Academic Research Journal, and she has been involved with Vertices since her freshman year. Upon graduation, Julia hopes to go to medical school with the intention of becoming an OBGYN. Julia also dances in Duke’s ballet Company (Devils en Pointe) and loves to play 70s and 80s blues songs on the electric guitar.

Sasha Bacot,
Editor-in-Chief

Sasha (Trinity ‘25) is from South Carolina and is a double major in Biology and Computer Science. She loves being a Vertices peer reviewer because it allows her to delve deeper into what she’s most passionate about: scientific research! Outside of her work with Vertices, Sasha loves to figure skate, listen to music, and try out all the cool restaurants in Durham (especially for boba)!

Kaeden Hill,
Editor-in-Chief

Kaeden (Trinity ‘25) is a Vertices Senior Editor from Atlanta, Georgia, double majoring in biology with a concentration in molecular and cell biology, and evolutionary anthropology with a minor in chemistry. After graduating, he plans to pursue a Ph.D. and a career in research. He is specifically interested in DNA tumor viruses and how their “cellular hijacking” can drive cells towards cancer, and he is a member of the Luftig Lab, studying Epstein-Barr virus and the cancers that it causes. Outside of academics, he loves to hike, travel, ski, scuba dive, collect minerals, and make jewelry.

Eliza Goldstein,
Senior Editor

Eliza Goldstein is a second-year undergraduate student majoring in Psychology with a minor in Global Health. She is interested in clinical and social psychology, specifically the intersections and variance of psychopathology among differing backgrounds and experiences. After completing her undergraduate degree, she plans to pursue a Ph.D. in Clinical Psychology. At Duke, she is the Lab Manager for the Culture Lab and a Research Assistant for the Zucker Lab. Outside of research, she enjoys biking, reading, and spending time with her friends.

Eric Lee,
Senior Editor

Eric is a junior from Jacksonville, Florida studying premedicine and computer science at Duke. He is particularly interested in the intersections of computational techniques and biomedical psychiatric research. On campus he is passionate about working with research education and mental health organizations. In his free time, he enjoys ping-pong, board/card games, cooking, and music.

Vishruth Hanumaihgari,
Senior Editor

Vishruth (Trinity ’27) is from Allentown, Pennsylvania and plans to major in neuroscience. He loves doing research in the lab, and he joined Vertices because he can read other people’s work and help them publish the best possible version of their projects. Outside of Vertices, Vishruth also loves to play basketball and try new foods!

PEER REVIEW TEAM

Aditya Raj

Aditya is a sophomore studying a combination of Biology, Information Technology, and Policy. He is passionate about regenerative medicine and scientific communication. He enjoys playing squash, walking in forests, and listening to jazz.

Angela Xiong

Angela Xiong is a first-year undergraduate student at Duke University, intending to major in Medical Sociology and minor in Chemistry. She is particularly drawn to the social determinants of health and addressing health disparities in minority and marginalized populations. Outside of Vertices, Angela also serves as a first-year senator on Duke Student Government, and volunteers at the puppy kindergarten!

Anya Milberg

Anya Milberg is a sophomore from New York City majoring in Neuroscience with a minor in Sociology. She is interested in exploring the intersection of science and law, currently researching trauma-informed court practices. Outside of Vertices, she can be found leading tours of campus, volunteering with the Duke Justice Project, baking, and spending time with friends.

Arielle Kim

Arielle Kim (Trinity ‘26) is a Duke University undergraduate majoring in Biology with minors in Computer Science and Computational Biology and Bioinformatics! She is particularly drawn to the intersection of microbiology and ecology, and she is currently exploring symbiotic systems involving fungi and their photobionts as a member of the Lutzoni Lab.

Arnav Singh

Arnav is a third-year undergraduate student in the Department of Biomedical Engineering. His passion lies at the intersection of AI and Medicine, and is currently working with Dr. Amanda Randles, on developing a Deep Learning Computational Fluid Dynamics (CFD) model for the noninvasive diagnosis of Coronary Artery Disease (CAD). Outside of Vertices, he serves as the Head of Duke Applied Recycling Center (DARC), the first on-campus plastic recycling center on a university campus in the United States.

Asher Wallen

Asher Wallen is a second-year Pratt student from Charleston, SC. He is majoring in biomedical engineering and computer science with a minor in French studies. He is also a member of Duke Brimstone, Duke Science Olympiad, Duke Robotics Mentorship, Duke STEM Connect, the Association of Mixed People at Duke, and Les Diables Bleus.

Caleb Lian

Caleb Lian is a freshman from San Marino, California majoring in Neurobiology and Psychology with a particular interest in psychopathology. He is currently exploring the role of epigenetic histone modifications on astrocyte maturation in the Eroglu Lab, and is also a part of the CAPER research program at the Duke Hospital. In his free time, Caleb enjoys running with DCR and playing intramural soccer.

Colby Cheshire

Colby is a senior studying Biology and French. He has previously worked with the Alberts Lab at Duke, the Speliotes Lab at the University of Michigan, and the Turnbaugh Lab at UCSF. Outside of lab and class, Colby enjoys volunteering with Crisis Text Line, reading books, and discovering new coffee shops in Durham.

Cooper Ruffing

Cooper is a Biology and Statistics Major from Raleigh, North Carolina. In addition to exploring his love for all sorts of sciences, he plays golf, volunteers with Duke STEM Connect, and tutors math!

Daliya Rizvi

Daliya Rizvi is a rising sophomore at Duke University, studying biochemistry and applied ethics on the premed track. Passionate about music, research, reading, and running track, Daliya prioritizes balancing her academic life with diverse interests. As a proud member of Vertices, Duke's premier research publication, Daliya is dedicated to showcasing the groundbreaking research conducted by fellow students.

Daniel Levin

Daniel is a first-year undergraduate from Pittsburgh, PA, studying biology and human rights. His research at Duke focuses on understanding the role of noncoding RNA during immune responses. After graduating, he aspires to pursue a Ph.D. to further investigate the mechanisms behind microbial pathogenesis and advance treatments for infectious diseases. Outside of the lab, Daniel enjoys biking and gardening.

Daniel Sul

I am a biology major with a chemistry minor on the premed track. On campus, I am involved in vision health research, clinical volunteering, and science journalism

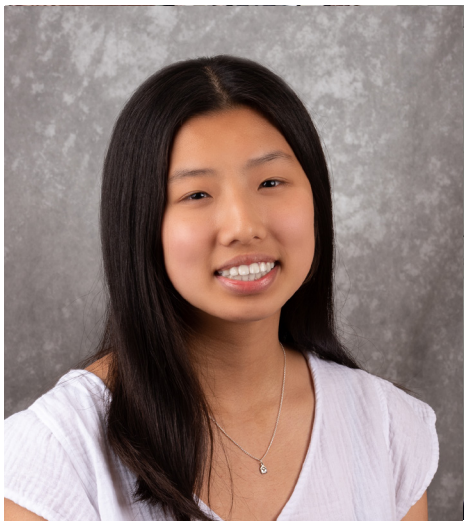
Darsh Mandera

Darsh is a first-year undergraduate with interests in AI, biology, and linguistics. His research experiences include work in precision oncology as well as computational modeling of stem cell differentiation. Beyond Vertices, he likes reading about language and current affairs.

Dennis Wu

Dennis Wu is a future astrophysicist working on research and fiction writing. He enjoys mountains as his refuge when he hikes and stargazes. As a peer reviewer at Vertices, he is dedicated to help students to bring forth quality research.

Grace Wang



Grace Wang is an undergraduate student intending to major in Chemistry or Evolutionary Anthropology. She is drawn to the puzzle-solving nature of scientific research and is currently exploring the organic synthesis of medicinal compounds in the Hong Lab.

Jane Lee

I am a sophomore majoring in biology with a neurobiology concentration with minors in psychology and chemistry. I'm originally from Southern California but now live in Cary, NC! My research interests include neurodegenerative disorders and developmental disabilities.

Jeffrey Bohrer

I am freshman from Guayaquil, Ecuador majoring in Chemistry with a concentration in chemical biology. I am currently working as a research assistant in Varghese Lab studying organ-on-chip platforms. Outside of academics, I am a huge fan of rock music!

Jeremy Yarden

Jeremy is a senior majoring in Biology with minors in Chemistry and Classical Civilizations. He currently works in the Kwatra Lab studying potential therapies for glioblastoma and is interested in pursuing a career in medicine.

Kate Lee

Kate Lee is a Duke undergraduate student from South Korea intending to major in either chemistry or biology. She is passionate about medicine and science and loves reading and writing scientific papers. She is currently studying cancer and immunotherapy, utilizing both germ free and SPF mice as a researcher in Conejo-Garcia Lab.

Katherine Long

Katherine Long (Trinity ‘24) is a Biology and Chemistry student with a passion for research and scientific communication. Outside of science, she enjoys painting, cooking, and reading.

Lizaveta Slootskaya

I am a current freshman studying Biomedical Engineering and Electrical Computer Engineering. I am very interested in cancer biology research and medical devices.

Nathanael Ren

Nathanael Ren is a student at Duke studying Computer Science, Electrical and Computer Engineering, and Mathematics. His previous work includes applications of the aforementioned fields to the financial services and healthcare fields. He is also involved in the Duke University Debate Society and Duke Robotics Team.

Nitya Chadha

My name is Nitya Chadha and I am a biology major and a peer reviewer for Vertices. Outside of school and extracurriculars, I love reading, singing, listening to music, and going for walks in my free time.

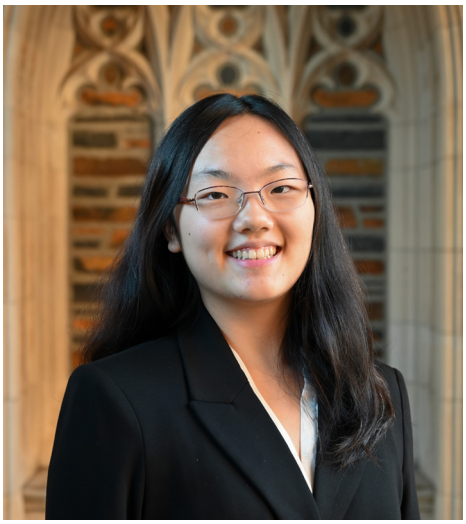
Sai Gayathri Kurup

I am a freshman in the Trinity College of Arts and Sciences, and I am a prospective biology major with a certificate in health policy. I am passionate about translational research, especially related to the life sciences, through the lens of science communication.

Will Sun

Will (Trinity ‘27) is an undergraduate student who intends to major in biology with a concentration in ecology, marine science and conservation from San Jose, CA. Outside of working in the Miao Lab on integrative immunology, he also enjoys playing basketball, watching sunsets, and eating ramen.

Zishen Li



Zishen is an undergraduate student at Duke. She is interested in medicine as well as the intersection between science and art. Outside of Vertices, she enjoys playing ping pong and trying out new things!

Meet Our Design Team

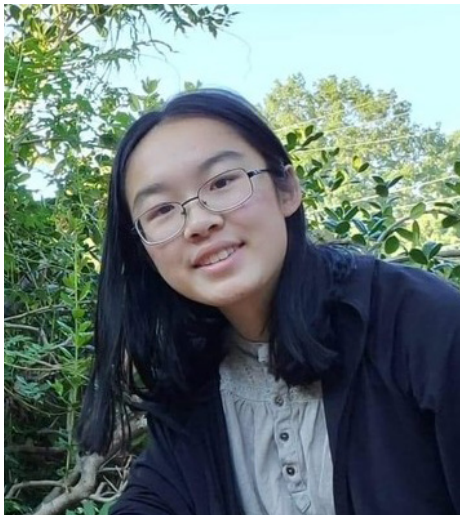
AJ Kochuba,
Artistic Director

AJ (Trinity ‘25) is from Cary, North Carolina, studying neuroscience, psychology, and visual arts on the pre-med track. AJ is particularly interested in humanities-based approaches to medical practice and research and hopes to enrich the symbiotic relationship between the fields of science and arts. Outside of Vertices, AJ can be found hosting arts- and identity-focused events and competing on the pickleball courts.

Sage Cooley
Website Director

Sage (Pratt ‘25) is from Raleigh, North Carolina and is majoring in mechanical / aerospace engineering. Outside of Vertices, you’ll find her working on rocketry with Duke’s AERO society, spending time with friends, or going on a nature walk!

Amanda Li



Amanda (Pratt ‘27) is from Charlotte, North Carolina and is majoring in biomedical engineering. She loves learning about anything and everything, doodling in her spare time, and exploring new things.

Cindy Ju

Cindy is a sophomore from South Carolina planning to major in economics. She enjoys arts and crafts, walking in the Duke Gardens, and trying out different boba shops around Durham.

Erin Heyeck

Erin (Trinity ‘24) is a Senior from Princeton, New Jersey, studying biology and computational biology. She is passionate about the intersections of science and art. Outside of academics, Erin can be found on the water with Duke Women’s Rowing and exploring new restaurants in the Triangle.

Gaby Dunn

Gaby is a sophomore from South Florida with too many interests that she is trying to find a major for. She is fascinated by the intersections of education, art, & STEM. When procrastinating, she enjoys rock climbing, critiquing movies, and spending time outdoors.

Jessica Pham

Jessica is a first year from Monroe, LA, planning to major in biomedical engineering and pursue the pre-med track. She's passionate about intellectual exploration, creative writing, and the intersection between science and the humanities. She may be found wandering various places, lost inside her head.

Monet Shum

Monet (Trinity '27) is a freshman from Aldie, Virginia planning to study linguistics, computer science, and classical civilizations. Outside of Vertices, she also works with Duke's Digital Art History and Visual Culture Lab and enjoys beading and walking in the Duke Gardens.

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