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Letter from the Editor

Dear Reader,

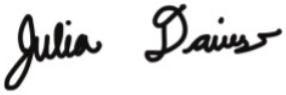
Welcome to the second issue of Vertices: Duke's Undergraduate Research Journal. This semester we expanded our editorial board to include over twenty peer reviewers and added Sasha Bascot and Kaeden Hill as Senior Editors. This second issue includes several enhancements: article synopses, reviewer biographies, and improved graphics and layouts thanks to our newly established design team.

Ultimately, this issue created opportunities for undergraduates to participate in all aspects of a rigorous journal from research and writing to reviewing and editing. Our editing team collaborated with Duke faculty members and Georgeotwn peer reviewers to produce a high quality research publication.

I'm honored to share this publication here. We chose to showcase five stellar articles carefully selected from all our submissions this semester. On the cover, we feature an article that provides insights into the efficiency of different methods for pluripotent stem cell differentiation, a research tool with the power to combat diseases from diabetes to childhood leukemia. Following this is a review of the Larock Indole Synthesis, which considers improvement to Larock's original work, leading to more significant pharmaceutical applications. Our third article builds upon the mental health mentoring article from our first issue, this time laying the groundwork for a more evidenced-based approach to mental health counseling at the university level. Next, article number four uses the Arc-Length Estimate formula to analyze the radiation from the Chernobyl Catastrophe, a tragic event in 1986 where radiation covered all of Pripyat, Ukraine. Finally, our last article calls for anti-gentrification policy change, backed by fMRI data measuring implicit racial bias in the amygdala (a part of the brain that controls the fear response).

I encourage you to join in my excitement as you read this thought-provoking publication from our 2022 Vertices team!

Sincerely,



Julia Davis, Editor in Chief

iPSC differentiation into various neuron subtypes

Emily Da Cruz

Volume 1, Issue 2

Article Synopsis

Induced pluripotent stem cells (iPSCs) are a valuable research tool to model diseases, serve as regenerative therapies, and to screen/test drug mechanisms; they are derived from an individual's blood or skin cells and reprogrammed into a state that will allow them to differentiate into any cell type. In the fields of neuroscience and neurology, iPSCs are of great interest in studying the various implications of neurological diseases on various neuronal cell types. Considering the derth of differing protocols for iPSC differentiation into neuronal types, it would be of value to consolidate said protocols to promote uniformity across studies utilizing iPSCs to study the nervous system. In this paper, we compile protocols for iPSC differentiation into various neuronal cell types and assess their timeline, efficacy, and required supplies in hopes that researchers can assess these protocols and select the best one for their aims, while maintaining a general consistency across the field.

Graphic by Erin Heyeck

iPSC differentiation into various neuron subtypes

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Highlights
- Review of iPSC differentiation methods for various neuronal subtypes
- Allows scientists to quickly reference various protocols that they can replicate in their own work
- Brief analysis comparing the efficiency of different methods, highlighting gaps in the current literature about iPSC differentiation

Abstract

Induced pluripotent stem cells (iPSCs) have been demonstrated to be a valuable tool in modeling diseases, investigating drug mechanisms, and understanding manifestations of disease in specific cell types. In the field of neurology, current studies are investigating the use of iPSCs as a regenerative therapy, which could be extremely beneficial for understanding and treating neurodegenerative diseases. While iPSCs have been used in various studies, a lack of familiarity with all available protocols for neuronal differentiation makes it difficult to identify best practices. Further, because neurological diseases can selectively impact certain types of neurons, it is useful to compile protocols to make different neuronal types. In this article, methods of differentiating iPSCs into various neuron subtypes are described in order to provide a consolidated list of protocols that will aid the analysis of suitable and available methods for specific research protocols. By compiling and summarizing these various methods, future studies can determine which methods are best for their specific research goals, regarding timing, efficiency, and accuracy.

Keywords: stem cells; iPSC; cell differentiation; neurons

I. Introduction

Cell manipulation is a prominent technique used to further understand the mechanisms of diseases, pathways of drug actions, and effects of gene therapies (Ferrari et al., 2020, Freel et al., 2020). Induced pluripotent stem cells (iPSCs) are a useful tool for cell manipulation, as they are stem cells that can be generated from somatic cells and give rise to cells in any of the three germ layers: the endoderm, ectoderm, or mesoderm. iPSCs have been vital to in-depth learning about various underlying mechanisms of disease through studying the cellular physiology in the cells with the patient's genetic background. In the field of neurology, this is particularly useful since human neuronal tissue is not as accessible to be studied as other human tissues, such as blood. Additionally, it is suggested that iPSCs can be used as a regenerative therapy to modify local pathophysiology in the brain and to screen potential drugs for neurological disorders. In short, the use of iPSCs in neuroscience and neurology-based research has the opportunity to expedite many of the processes involved in research regarding rare/complex disorders as they allow for specificity that is second only to studying the actual patient. In this way, a patient can be protected from any potential adverse effects of research but still have treatments that are tested on their specific genetic background in cells that originate directly from them.

While the use of iPSCs show potential for the future, a lack of a consolidated compilation of the protocols used to differentiate iPSCs into various

types can lead to difficulties in planning for studies or missing useful alternative methods. In this article, we review and present the differentiation methods used for generating various neuron subtypes, both in written and table form (see Table 1). It is without question that the implementation of differing methods can lead to drastically different results; for example, two studies evaluating the role of the prion protein as a receptor for amyloid-β in a possible mechanism for Alzheimer's disease presented conflicting results, largely explained by their differences in protocols (Laurén et. al., 2009; Kessels et. al., 2010). Developing a uniform collection of protocols for differentiating iPSCs into neuronal types should greatly benefit the fields of neuroscience and neurology. Researchers interested in utilizing iPSCs to evaluate a specific neuronal cell type need standardized methods that not only allow them to feel confident that they will successfully generate the neuronal cell types they desire but also allow them to generate transferable results that are not limited by shortcomings that may exist. It is the aim of this paper that by compiling and briefly presenting these various methods, future studies can not only determine which methods are best for their specific research goals, but also utilize uniform methods to increase the cohesiveness and consistency of findings across studies within the field.

II. Historical Background

Induced pluripotent stem cells were first generated in 2006 using four transcription factors, Oct3/4, Sox2, c-Myc, and Klf4 (now coined "Yamanaka factors"), in mouse embryonic or adult fibroblasts (Takahashi and Yamanaka, 2006). These iPSCs, with the ability to differentiate into an array of cell subtypes in vitro and in vivo, have expanded the manipulation of pluripotent cells and changed the manner in which cell types can be analyzed and studied. With all four Yamanaka factors, iPSCs are identical in pluripotency stem cells but are tumorigenic. The discovery that the tumorigenicity could be reduced by removing the c-Myc transcription factor was instrumental in making iPSCs safe and practical for today's clinical applications (Nagawaka et al., 2008). iPSCs have been vital tools in research on disease mechanisms, regenerative cell therapies, and drug mechanisms. (Aoi et. al., 2016). iPSC-based disease modeling has been particularly useful for genetic disease research, as patient-derived iPSCs can be used to study the impacts of genetic mutations on various cell types (Shi et. al., 2017). Furthermore, the potential use of iPSCs in regenerative therapies has been particularly important in the studies of Parkinson's disease, spinal cord injuries, liver dysfunction, and more (Aoi et. al., 2016). Despite the immense progress that has been made in the biomedical field thus far due to iPSCs, there are still gaps in the knowledge of iPSCs in vivo that must be understood in order to optimize their use for translational applications. This, in part is due lack of consistent quality and streamlined differentiation protocols and materials (Liu et. al., 2020). Thus, a review, like the one presented here, that compiles and presents these methods would be helpful by providing a comprehensive source for various such protocols.

III. Neuron Subtype Generation from iPSCs

In this section, the details for various protocol methods are described. The methods are organized based on the type of neuron the iPSCs ultimately differentiate into: glutamatergic, GABAergic (medium spiny neurons, Purkinje neurons, and Parvalbumin-positive interneurons), dopaminergic, serotonergic, cholinergic, and PNS (sensory and autonomic nervous system) neurons. Of note, some of the key ways in which these protocols will achieve differentiation of the iPSCs is by physical manipulation of the cells (i.e. growing them in neurospheres), adding small molecules to the mediums a being used (e.g. addition of ROCK inhibitor), and expression of fate-determining transcription factors (e.g. Ngn2 transient overexpression). While transcription factor expression is typically the most efficient of the three, depending on the neuronal type to be differentiated or the goals of the researcher, other methods may be more beneficial, in terms of their timeline, efficacy, and costs. By providing details for such protocols, regarding the mediums' compositions, timelines, and efficacy, as well as methods to confirm the identity of the fully differentiated cells at the end of the procedure, we hope to provide an objective presentation of the protocols that have proven to be valuable in creating such neuronal cell types in order to help provide direction for researchers interested in

using iPSCs in their work.

1) Glutamatergic

Begum's methods of differentiation: Three different methods have been used in the past to differentiate iPSCs into excitatory glutamatergic neurons. The first method utilizes neurospheres (also known as progenitor cells) as an intermediate between iPSCs and glutamatergic neurons. Once iPSC colonies are formed, they are treated with collagenase IV to initiate neural differentiation. After being harvested and resuspended in a knockout serum replacement medium, the culture is plated on low adhesion suspension culture plate in a broth for approximately 3 days in order to generate the neurospheres, which are maintained in neuronal induction medium (NIM). The neurospheres are then collected and broken down into fragments on Matrigel-coated plates, leading to the formation of a neuroepithelial sheet known as a "neurosphederm." This phase is followed by a 3–5-day incubation period which allows neural progenitors to form, thus preparing the culture for sub-type and region-specific differentiation. Neuronal Maintenance Medium (NMM) is then used in the plates until glutamatergic neurons are generated (roughly one week after neuronal induction, around day 17). This method has proved to be more efficient than pre-existing ones, specifically the neuroectoderm method, and studies utilizing this method showed a doubling in expression of neuroprogenitor genes (Begum et. al. 2015).

Shi's method of differentiation: Alternatively, Shi et. al. (2012) provides an extensive protocol to generate glutamatergic neurons from iPSCs. To start, iPSCs are cultured by plating mouse embryonic fibroblasts (MEFs) in human pluripotent stem cell (hPSC) medium and repeatedly freezing, thawing, and resuspending the culture until colonies appear. Colonies are occasionally fragmented to encourage growth. With the iPSCs now prepared for differentiation, neuronal induction can occur once colonies are plated with a neural induction medium and then incubated for 8-12 days. After the incubation period, a neuroepithelial sheet appears, which then needs to be fragmented by pipetting. The plated culture is then put in a neural maintenance medium which leads to the formation of neural rosettes. The neural rosettes are then centrifuged and resuspended in medium frequently over 20 to 30 days for neurogenesis to occur. Functional glutamatergic synapses typically arise within 30-50 days. Differentiation into glutamatergic neurons comes with the loss of expression of pluripotency genes and expression of transcription factors and proteins associated with excitatory glutamatergic neurons (vGlut1 and PSD-95). Shi et. al. explains that the efficiency of this method is dependent upon the pluripotent stem cells used in differentiation, but can approach 100%.

Wang's method of differentiation: Wang et. al. (2017) described a two-step protocol for differentiating iPSCs into glutamatergic neurons. Namely, this protocol leads to transiently overexpressing a transcription factor, neurogenin 2 transgene (Ngn2). The transient induction of lentivirus-mediated Ngn2 is achieved by delivering the transcription factors to AAV integration site 1 safe-harbor locus induced using doxycycline incubation for 3 days; the second (and final step) for this method is subplating of the post-mitotic resultant cells with a specified medium containing N2 and B27 supplements. Within 7 days, neuron morphology is observed and mature morphology in the absence of glia is observed within 3 to 4 weeks. Further confirmation of the functionality of these newly differentiated neurons is confirmed by the expression of vesicular glutamate transporter 1 (vGLUT1) and by being GABA-negative, as indicated by the absence of GABA-positive inhibitory neurons by immunocytochemical staining analysis. Importantly, the efficiency of this method is >90%.

Methods to identify glutamatergic transformation: While the processes detailed by Shi et. al. (2012) and Wang et. al. (2017) are certainly longer than that of Begum et. al. (2015), they all lead to robust neuron populations differentiated into glutamatergic neurons, with differing advantages depending on the personal goals of the researcher. After differentiating the iPSCs, one can confirm the presence of glutamatergic neurons using immunofluorescence staining with the antibodies associated with this neuron type: vGlut1, PSD-95, and Homer1. If the cells viewed are tagged with the appropriate immunofluorescence stain, it

can be confirmed that glutamatergic neurons were differentiated from the iPSC cultures.

2) GABAergic

a) Medium Spiny Neuron (MSN) Differentiation

The mechanism described by Begum et. al. (2015) the glutamatergic differentiation process can also be applied to GABAergic neurons, with their appearance occurring a week after neuronal induction. Considering this method has already been described, the protocol will not be repeated in this section.

Grigor'eva's method of differentiation: One possible method for GABAergic differentiation is using Grigor'eva et. al.'s (2020) protocol for a specific subtype of GABAergic neurons: medium spiny neurons (MSNs). This method utilizes ROCK inhibitor in its medium to ultimately produce MSNs. iPSCs at 70-80% confluency are plated in neuronal differentiation medium for five days. In the following 7 days, the neuronal differentiation medium should not include SB431542 inhibitor or dorsomorphin. At day 12, the neuroectodermal cells in the medium are disaggregated and then replated with the same medium used previously but supplemented with ROCK inhibitor. Two days later, the medium should change again to a 1:1 ratio of the current medium:NeuroB (which includes B27 and recombinant human brain derived neurotrophic factor). The medium will transition fully to NeuroB on day 15 and cell culturing forms a monolayer that is maintained every 7-10 days. At this point, the precursor to MSNs is fully formed. To generate mature MSNs, the culture is plated in a NeuroBC medium (similar to the prior medium, but now containing recombinant human CTNF) for 10-20 days, with the medium being refreshed every other day. At this point, MSNs are fully generated and ready for functional use.

Methods to identify GABAergic MSN transformation: When testing to identify the presence of GABAergic neurons, immunofluorescence staining followed by microscopy is used to identify the neurons. Using GAD65, GAD67, anti-GAD6, and VGat antibody stains on the differentiated neurons, identifying the presence of GABAergic neurons is clear (Begum et. al. 2015 & Zhang et. al. 2013). More specifically, to confirm the presence of MSNs, Grigor'eva et. al. (2020) stained terminally differentiated MSNs with TUJ1, GABA, and NF200 antibodies. Upon visualization, these markers confirm the presence of MSNs differentiated from iPSCs. Neurons that exhibit immunofluorescence from the aforementioned antibodies can be confirmed as GABAergic neurons, and, in the case of Grigor'eva et. al. (2020), medium spiny neurons.

b) GABAergic Purkinje Differentiation

Begum's method of differentiation and identification of transformation: Purkinje neurons, a subtype of inhibitory neurons located in the cerebellar cortex of vertebrates, can be differentiated using the same process by which glutamatergic and general GABAergic neurons can be differentiated, based on the findings of Begum et. al. (2015). Once the progenitors are cultured from the iPSCs and placed in neuronal maintenance medium (NMM), cerebellar Purkinje neurons form approximately 40-45 days later, given that additional supplements are added to the medium, although the details of such supplements are not provided. Upon developing sufficient quantities of Purkinje neurons, immunofluorescence imaging can once again be used to confirm the presence of these neurons. Calbindin, the marker most tightly associated with Purkinje neurons, can be identified in the differentiated cultures during confocal microscopy.

c) GABAergic Parvalbumin-Positive Interneuron Differentiation

Maroof's method of differentiation and identification of transformation: Maroof et al. (2013) demonstrated how parvalbumin-positive cortical interneurons can be differentiated through pluripotent stem cells using a mouse cortical coculture strategy. Human embryonic stem cells (hESCs) and iPSCs are placed in a differentiation medium that consists of (1) knockout serum replacer, (2) N2 medium for neural induction, (3) neurobasal medium +B27, and (4) N2 supplements for neuronal differentiation. Following this, recombinant growth factors (SHH, Noggin, DKK1, BDNF, and FGF2) are introduced to promote proper

differentiation. Using the coculture system allows for rapid maturation of neurons and enables the expression of markers associated with the cortical interneuron subtype, specifically parvalbumin and somatostatin. In order to confirm the presence of parvalbumin-positive interneurons, cells are co-labeled with calbindin antibody, a calcium-binding protein co-expressed by parvalbumin-positive interneurons, and analyzed with Operetta, an automated high content imaging system; it was found that approximately 5% of GFP stained cells had high expression of parvalbumin and interneuron-morphology. This immunohistochemistry analysis confirms the presence of parvalbumin-positive interneurons because cells that indicate expression of calbindin indicates properly differentiated cells.

3) Dopaminergic

Xue's method of differentiation: Xue et al. (2019) describes a method in which differentiation of midbrain dopaminergic neurons is accomplished in a five-day time period using iPSCs and mRNA coding for two proneural transcription factors: Atoh1 and Ngn2. The iPSCs are plated with growth-factor reduced Matrigel and then transfected with Atoh1 mRNA for 3 days and Ngn mRNA for 1 day. The culture medium contains SHH, FGF8b, and DAPT. After the five-day period, the medium is changed to a neurobasal medium with B27 supplement, BDNF, GDNF, TGFbeta-3, cAMP, ascorbic acid, and DAPT. Regular changing of the medium is useful to remove unattached cells and promote maturation into midbrain dopaminergic neurons.

Method to identify dopaminergic transformation: In order to confirm the presence of midbrain dopaminergic neurons, Xue et al. stained neurons with primary antibodies, LMX1A, DAT, TH, Nurr1, and GIRK2 and conducted immunofluorescence imaging to determine if the cultured cells expressed the proteins associated with dopaminergic neurons. Expression of these antibodies is indicative of a successful differentiation into midbrain dopaminergic neurons.

4) Serotonergic

Serotonergic neurons have been generated from iPSCs, utilizing a procedure that involves human fibroblasts. Xu et. al. (2016) and Vadodaria et. al. (2016) both successfully used this method to form functional serotonergic neurons that they explain are promising in the context of studying selective serotonin reuptake inhibitors (SSRIs) and serotonin-related mental disorders.

Vadodaria's method of differentiation: Vadodaria et. al. (2016) demonstrated that human dermal fibroblasts can be used to generate iPSCs that can then be differentiated into fully functional serotonergic neurons. The fibroblasts undergo transduction with lentiviral particles for pLVXEtO,39 ASCL1/NGN2, and induced serotonergic neuron factors. Following an incubation of approximately 24 hours, the transgenic-induced neuron and induced serotonergic neuron-competent fibroblasts are transferred into a tetracycline-free FBS-containing medium. An ASCL1/NGN2-based protocol is used for direct neuronal transdifferentiation and then fibroblasts are plated with induced neuron conversion medium for three weeks. After this time period, the medium is changed to neural maturation medium to further mature the induced neurons. The entire process takes roughly 3-6 weeks and produces cultures of serotonergic neurons.

Xu's method of differentiation: In the protocol designed in Xu et. al. (2016), serotonergic neurons are also induced from human fibroblasts, specifically MRC5, CCD-19Lu, and IMR90, that are maintained in DMEM. Cotransfecting 293FT cells with FUW-tetO-LoxP-cDN formed the lentivirus. This is then used to infect the fibroblasts to change their morphology to appear more like neurons. After 16 hours, the medium infected with lentivirus is replaced with new DMEM, followed by neural induction medium 24 hours later. Doxycycline is included in the medium for the first seven days, and dorsomorphin and SB431542 are included on days 2 through 7. Until sufficient cultures, as determined by the scientist, are generated, the medium must be replenished every other day. The overall timeline, from plating to generation of cultures, is approximately 15 days.

Methods to identify serotonergic neuron transformation: Immunofluorescence imaging of

the serotonergic cells created is used in both Xu et. al. (2016) and Vadodaria et. al. (2016) in order to confirm the identity of the cells cultured. The antibody, 5-HT, is used in both studies to stain the neurons and serve as an indicator of the presence of serotonergic neurons.

5) Cholinergic

Begum's method of differentiation: Begum et. al.'s method of differentiation through neurospheres is also applicable to the generation of cholinergic neurons, although cholinergic neurons do have some key differences to their methods than other neuron types. Importantly, cholinergic neurons form in the NMM in 19-27 days, much faster than other neuron types, such as Purkinje neurons, which require 40-45 days in the NMM before generation. All steps leading up to the generation of cholinergic neurons based on the Begum et. al. (2015) protocol remain the same.

Takayama's method of differentiation: Alternatively, Takayama et. al. (2020) also successfully generated cholinergic neurons from iPSCs using a stepwise chemical induction method. The iPSC lines must be maintained in a medium for up to three days to allow for the generation of embryonic bodies. These embryonic bodies are then cultured in knockout serum replacement for 13 days (with routine changing of the medium) before being plated in a new neuronal differentiation medium containing N2 medium, forskolin, ascorbic acid, recombinant human brain-derived neurotrophic factor, recombinant human glial-cell-derived neurotrophic factor, recombinant human nerve growth factor, and recombinant human neurotrophin 3. Recombinant human ciliary neurotrophic factor is also present in the medium and assists with the differentiation into parasympathetic neurons. The medium is changed every two weeks until cholinergic neuron colonies form. A key point to consider is the cholinergic neurons are derived from a specific neural crest; Takayama's method utilizes a neural crest induction step followed by an autonomous specification step with restricted Wnt signaling inhibition, Sonic Hedgehog (Shh) signaling inhibition, and Bone Morphogenic Protein (BMP) signaling activation which helps guide the differentiation to specific lineages to ensure cholinergic neurons are derived.

Methods to identify cholinergic neuron transformation: Immunofluorescence imaging with antibody tagging is, once again, the most effective way to determine if the cultured cells were properly differentiated, due to its ease in use and specificity. Begum and Takayama explain that the two antibody tags that are most tightly linked to cholinergic expression are CHAT and nAchR. Cells exhibiting immunofluorescence after staining with these two antibodies can be considered cholinergic neurons with successful gene expression. Confocal microscopy is often used to visualize the antibody stains (Begum et. al. 2015 and Takayama et. al. 2020).

6) Peripheral

a) Sensory Neurons

Chambers' method of differentiation: The generation of sensory neurons from iPSCs has been successfully completed by Chambers et. al. (2012). Perhaps most exciting regarding Chambers' method is that it is a notably fast protocol, yet still efficient in generating differentiated neurons. Using a combination of 5 small-molecule pathway inhibitors, Chambers was able to differentiate mature and effective neurons in approximately 10 days. The combination utilized by Chambers, containing SU5402, CHIR99021 and DAPT, eliminates the expression of PAX6, which is the human neuroectoderm marker, and induces neuronal β3-tubulin. Additionally, two other inhibitors, LDN-193189 and SB431542, replace Noggin and neutralize pluripotent stem cells. These 5 inhibitors, when used together (termed the LSB3i treatment), lead to efficient maturation of neurons generated from iPSCs using dual-SMAD inhibition. While all three major types of sensory neurons are generated (proprioceptors, mechanoreceptors, and nociceptors) more than half of those generated in Chambers' study (approximately 60%) were nociceptors. Within 10 days, sensory neurons can be effectively harvested.

Methods to identify sensory neuron transformation: Sensory neuron generation can be confirmed using immunofluorescence staining and imaging. General

sensory neurons can be identified by staining the cells with BRN3A and ISL1 antibodies after day 11. Microscopy images showing stained cells expressing the antibody indicate that the cells generated are functioning sensory neurons. Chambers et. al. focused their studies specifically on nociceptor neurons, as they are the most plentiful form of sensory neurons derived from this method. Because of this, antibody stains specific to nociceptors can also be used to further confirm the subtype of sensory neuron derived. The following antibody tags can be used to identify nociceptor sensory neurons: RUNX1, RET, SCN10A, P2RX3, and TPVR1. Positive expression of these antibodies in imaging indicates that the cells tagged are successfully differentiated nociceptor sensory neurons.

b) Autonomic Nervous System Neurons

Saito-Diaz's method of differentiation: Postganglionic sympathetic neurons of the peripheral nervous system have been successfully differentiated from iPSCs using the protocol developed in Saito-Diaz et. al. (2019). This protocol can be divided into three key steps: developing neural crest cells, culturing as neural crest spheroids, and long-term differentiation. To form the neural crest cells, iPSCs are plated with a neural induction cocktail that activates and inhibits specific pathways necessary for neural crest cells. After approximately 11 days, the neural crest cells are isolated and fluorescence-activated cell sorting is used to purify them, keeping the SOX10/CD49D-positive cells to ultimately form neural crest spheroids. The spheroids are suspended in neural crest spheroid medium and be prepared for differentiation by day 15 of the process. At this point, the spheroids are replated on poly-L-ornithine, laminin, and fibronectin (PO/LM/FN)-coated plates in autonomic neuron medium until at least day 19. After day 19, postganglionic neurons are differentiated, however, it is still possible to replate the spheroids on PO/LM/FN-coated plates on day 20 with the autonomic neuron medium and further mature the neurons.

Methods to identify autonomic nervous system postganglionic neuron transformation: Saito-Diaz references Zeltner et. al. (2016) when discussing the method for characterizing the differentiated neurons using their protocol. Zeltner et. al. (2016) clearly shows that immunostaining the cells and visualizing them with microscopy methods allows for clear characterization of cells. Specifically, sympathetic autonomic neurons can be identified when marked with ASCL1, PHOX2A, SCG10 (STMN2), TH and DBH, all of which are antibody markers associated with autonomic neurons. Using this method, one can easily characterize the differentiated cells to ensure that they are sympathetic autonomic neurons.

IV. Conclusions

The various differentiation protocols highlight the diversity of processes that can be utilized to achieve each respective neuron subtype. Importantly, the variety in methods suggests that different protocols may be better for different types of studies; for example, a more time-sensitive study may benefit from shorter differentiation processes, described in Chambers et. al. (2012). Alternatively, methods that are more time-consuming, such as Begum et. al.'s (2015) 45-day process for differentiating Purkinje cells, should be assessed more analytically: does the lengthy process producing differentiated cells that are of significantly better quality than faster methods? One of the major limitations in comparing the various differentiation protocols aforementioned is the fact that there is no standardized method to quantify the efficiency of these protocols in comparison to each other and to non-iPSC-transformed corresponding types of neurons. There is thus need for standardized protocols for such comparisons using single cell sequencing, which will provide information regarding gene expression and can be compared not only to the data of other researchers but also to datasets from the brain to map the cultured cells onto the most similar cell type in the brain. It is difficult to perform a cost-benefit analysis comparing these studies at this point due to the lack of uniformity in the data presented by each paper. Future studies hoping to finetune the efficiency and quality of differentiating iPSCs into neuronal subtypes could compare the various methods described here, focusing on assessing methods for differentiation into the same type of neuron subgroup, as well as comparing across neuron subgroups in order to have a more holistic understanding of the merits of these various

methods in comparison to one another. The use of immunofluorescence imaging was uniform across all methods to confirm the presence of the differentiated neurons, suggesting this is an accurate and valuable method to confidently confirm the identity of the cell that has been differentiated. By analyzing the commonalities across protocols for neuron subtypes, it is possible for researchers to determine which methods are most suitable to their specific goals.

There are no conflicts of interest associated with the production of this manuscript.

Table 1:

Neuron Subtype Generation and Protocol Source	
Glutamatergic Neurons	Begum et. al. (2015); Shi et. al. (2012); Wang et. al. (2017)
GABAergic Medium Spiny Neurons	Grigor'eva et. al. (2020)
GABAergic Purkinje Neurons	Begum et. al. (2015)
GABAergic Parvalbumin-Positive Interneurons	Maroof et. al. (2013)
Dopaminergic Neurons	Xue et. al. (2019)
Serotonergic Neurons	Vadodaria et. al. (2016); Xu et. al. (2016)
Cholinergic Neurons	Begum et. al. (2015); Takayama et. al. (2020)
Peripheral Sensory Neurons	Chambers et. al. (2012)
Peripheral Autonomic Nervous System Neurons	Saito-Diaz et. al. (2019)

References

1. Aoi T. 10th anniversary of iPS cells: the challenges that lie ahead. *J Biochem*. 2016;160(3):121- 129. doi:10.1093/jb/mvw044
2. Begum AN, Guoynes C, Cho J, Hao J, Lutfy K, Hong Y. Rapid generation of sub-type, region-specific neurons and neural networks from human pluripotent stem cell-derived neurospheres. *Stem Cell Res*. 2015;15(3):731-741. doi:10.1016/j.scr.2015.10.014
3. Chambers SM, Qi Y, Mica Y, et al. Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors. *Nat Biotechnol*. 2012;30(7):715-720. Published 2012 Jul 1. doi:10.1038/nbt.2249
4. Ferrari E, Cardinale A, Picconi B, Gardoni F. From cell lines to pluripotent stem cells for modelling Parkinson's Disease. J Neurosci Methods. 2020 Jul 1;340:108741. doi: 10.1016/j.jneumeth.2020.108741. Epub 2020 Apr 18. PMID: 32311374.
5. Freel BA, Sheets JN, Francis KR. iPSC modeling of rare pediatric disorders. J Neurosci Methods. 2020 Feb 15;332:108533. doi: 10.1016/j.jneumeth.2019.108533. Epub 2019 Dec 4. Erratum in: J Neurosci Methods. 2020 Jun 1;339:108739. PMID:31811832; PMCID: PMC7310918.
6. Grigor'eva EV, Malankhanova TB, Surumbayeva A, et al. Generation of GABAergic striatal neurons by a novel iPSC differentiation protocol enabling scalability and cryopreservation of progenitor cells. *Cytotechnology*. 2020;72(5):649-663. doi:10.1007/s10616-020-00406-7
7. Kessels HW, Nguyen LN, Nabavi S, Malinow R. The prion protein as a receptor for amyloid-beta.

Nature. 2010;466(7308):E3-E5. doi:10.1038/nature09217

8. Laurén J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM. Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature*. 2009;457(7233):1128-1132. doi:10.1038/nature07761
9. Liu G, David BT, Trawczynski M, Fessler RG. Advances in Pluripotent Stem Cells: History, Mechanisms, Technologies, and Applications. *Stem Cell Rev Rep*. 2020;16(1):3-32. doi:10.1007/s12015-019-09935-x
10. Maroof AM, Keros S, Tyson JA, et al. Directed differentiation and functional maturation of cortical interneurons from human embryonic stem cells. *Cell Stem Cell*. 2013;12(5):559- 572. doi:10.1016/j.stem.2013.04.008
11. Nakagawa M, Koyanagi M, Tanabe K, et al. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol*. 2008;26(1):101-106. doi:10.1038/nbt1374
12. Nicholas CR, Chen J, Tang Y, et al. Functional maturation of hPSC-derived forebrain interneurons requires an extended timeline and mimics human neural development. *Cell Stem Cell*. 2013;12(5):573-586. doi:10.1016/j.stem.2013.04.005
13. Saito-Diaz K, Wu HF, Zeltner N. Autonomic Neurons with Sympathetic Character Derived From Human Pluripotent Stem Cells. *Curr Protoc Stem Cell Biol*. 2019;49(1):e78. doi:10.1002/cpsc.78
14. Shi Y, Inoue H, Wu JC, Yamanaka S. Induced pluripotent stem cell technology: a decade of progress. *Nat Rev Drug Discov*. 2017;16(2):115-130. doi:10.1038/nrd.2016.245
15. Shi Y, Kirwan P, Livesey FJ. Directed differentiation of human pluripotent stem cells to cerebral cortex neurons and neural networks. *Nat Protoc*. 2012;7(10):1836-1846. doi:10.1038/nprot.2012.116
16. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126(4):663-676. doi:10.1016/j.cell.2006.07.024
17. Takayama Y, Kushige H, Akagi Y, Suzuki Y, Kumagai Y, Kida YS. Selective Induction of Human Autonomic Neurons Enables Precise Control of Cardiomyocyte Beating. *Sci Rep*. 2020;10(1):9464. Published 2020 Jun 11. doi:10.1038/s41598-020-66303-3
18. Vadodaria KC, Mertens J, Paquola A, et al. Generation of functional human serotonergic neurons from fibroblasts. *Mol Psychiatry*. 2016;21(1):49-61. doi:10.1038/mp.2015.161
19. Wang C, Ward ME, Chen R, et al. Scalable Production of iPSC-Derived Human Neurons to Identify Tau-Lowering Compounds by High-Content Screening. *Stem Cell Reports*. 2017;9(4):1221-1233. doi:10.1016/j.stemcr.2017.08.019
20. Xu Z, Jiang H, Zhong P, Yan Z, Chen S, Feng J. Direct conversion of human fibroblasts to induced serotonergic neurons. *Mol Psychiatry*. 2016;21(1):62-70. doi:10.1038/mp.2015.101
21. Xue Y, Zhan X, Sun S, et al. Synthetic mRNAs Drive Highly Efficient iPS Cell Differentiation to Dopaminergic Neurons. *Stem Cells Transl Med*. 2019;8(2):112-123. doi:10.1002/sctm.18-0036
22. Zeltner N, Fattahi F, Dubois NC, et al. Capturing the biology of disease severity in a PSC-based model of familial dysautonomia. *Nat Med*. 2016;22(12):1421-1427. doi:10.1038/nm.4220
23. Zhang Y, Pak C, Han Y, et al. Rapid single-step induction of functional neurons from human pluripotent stem cells. *Neuron*. 2013;78(5):785-798. doi:10.1016/j.neuron.2013.05.029

Application of Palladium Chemistry in Organic Synthesis Techniques

Angikar Ghosal

Volume 1, Issue 2

Article Synopsis

The Larock Indole Synthesis is a method, discovered by Richard Larock in 1991, to produce indole, an important biological molecule. In this literature review, Ghosal talks about the historical overview, the advantages of the Larock Indole synthesis over earlier techniques, and the mechanism of the reaction. Ghosal also elaborates the various applications of this technique in organic chemistry.

Application of Palladium Chemistry in Organic Synthesis Techniques: the Larock Indole Synthesis, a Literature Review

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Abstract

As one of the most important biologically active and naturally occurring molecules, indole is an organic compound of great importance. For more than a century, the standard method of lab preparation of the indole family of compounds has been the Fischer indole synthesis. In 1994, Richard C. Larock and Eul Kgun Yum prepared indole and indole derivatives using a new technique involving organometallic chemistry, what has since then come to be known as the Larock indole synthesis [1]. In this literature review, we give a background to the techniques involved in the Larock indole synthesis, including similar precursor methods in palladium chemistry. We mention the motivations for this reaction method, based on earlier work done. We then give a detailed overview of Larock's work, including the reaction mechanism, kinetic effects, and effects of substituents. We also consider limitations of the Larock indole synthesis. We then study the various applications of this synthesis technique, especially synthesis of those compounds with pharmaceutical applications. We also then consider improvement to Larock's original work, including newer, more improved techniques that built on Larock's work, and how these newer discoveries are more beneficial to us. We end this review paper with some future questions to pursue.

Keywords: larock indole synthesis, organic synthesis, indole, palladium chemistry, organometallic chemistry

Relevance and Justification

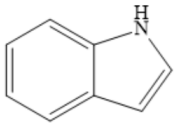

Fig 1a: Indole

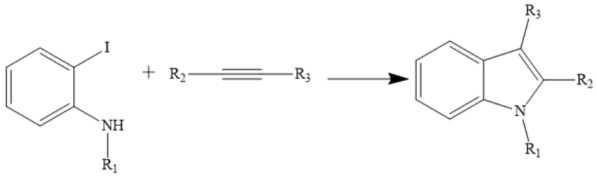

Fig 1b: Overall Reaction in Larock Indole Synthesis

Indole (Fig 1a) is one of the most important biologically active molecules and is a key example of an aromatic heterocyclic compound, composed of two rings. For example, it is a biological precursor to the synthesis of the essential amino acid tryptophan, which is also crucial to the synthesis of the neurotransmitter serotonin. After tryptophan is converted into 5-HTP, it is converted into serotonin, a neurotransmitter that relays signals between brain cells. 5-HTP dietary supplements help raise serotonin levels in the brain. Thus, effective laboratory and industrial preparation of indole has been a significant achievement in organic chemistry.

History and Introduction:

Fischer Indole Synthesis: Overview and Limitations

One of the earliest syntheses of indole is the landmark Fischer indole synthesis (Fig 2), which used phenylhydrazines [2] as the starting product.

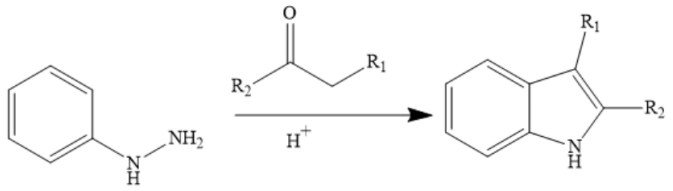

Fig 2: Fischer Indole Synthesis

This reaction involves condensation to form a phenylhydrazone, then isomerization to an enamine, a cyclic [3,3] sigmatropic rearrangement to form an imine. The imine then cyclizes to a cyclic aminoacetal, which then eliminates ammonia under acidic workup. As the final elimination involves an acidic workup, this reaction can be catalyzed by acids such as p-toluenesulphonic acid or even Lewis acids such as zinc chloride or aluminium chloride.

This reaction mechanism is classical, with the condensation-rearrangement-cyclization steps. As is described later, such classical techniques have some limitations which could only be overcome with the development of organometallic chemistry, including techniques such as the Larock indole synthesis. The Fischer synthesis came out in 1884 [2], while the Larock synthesis came out in 1991 [1], more than a hundred years later.

The methodologies used in the Fischer indole synthesis were soon used to synthesize other heterocyclic aromatic compounds, including the Borsche-Drechsel synthesis of carbazole [3]. Carbazole has been used to manufacture pigments, as well as carprofen, a non-steroidal anti-inflammatory drug of the propionic acid class. The broad techniques used in this class of reactions involved ring formation by dehydrogenation, and this is used to manufacture a series of compounds in the first half of the 19th century, which involved an indole nucleus and multiple rings.[4] (Fig 3)

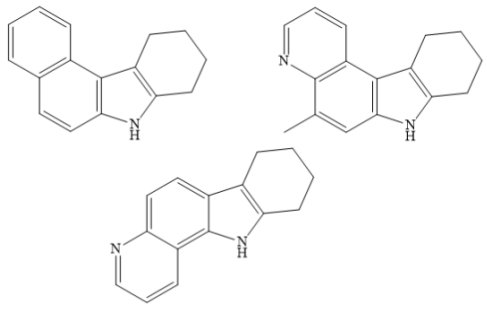

Fig 3: Compounds Prepared using Fischer Indole Synthesis

However, there are multiple issues with this method of synthesis [4].

1. For heavier organic compounds, the yield is extremely low, with the dehydrogenated final product found as intractable tars.
2. Over-dehydrogenation cannot be controlled, as the use of an acidic catalyst often meant a fully aromatized compound is prepared, instead of just the required indolized aromatic nucleus.
3. Furthermore, attempts were made to make the Fischer indole synthesis more dynamic and flexible by considering substituted phenylhydrazones, but these fail in many cases. For example, both 2-ketoester phenylhydrazones and 3-ketoacid phenylhydrazones fail to cyclize and form the aromatic indole nucleus. 2-ketoester phenylhydrazines tend to produce pyrazolones, while 3-ketoacid phenylhydrazines form pyridazinones (Fig 4). Similar results are obtained for other substituted phenylhydrazines. As this article later explores, these issues can be solved by using the Larock indole synthesis.

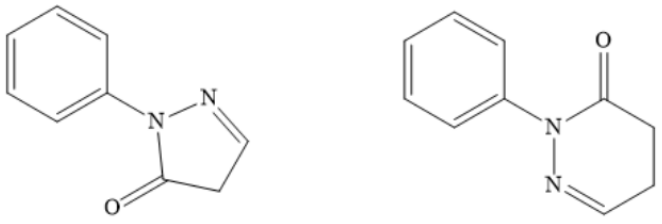

Fig 4: pyrazolone (left), pyridazinones (right)

4. Similarly, indolization of pyridylhydrazones and pyrimidylhydrazones have failed [5], with other

heterocyclic aromatic compounds being produced as by-products (Fig 5).

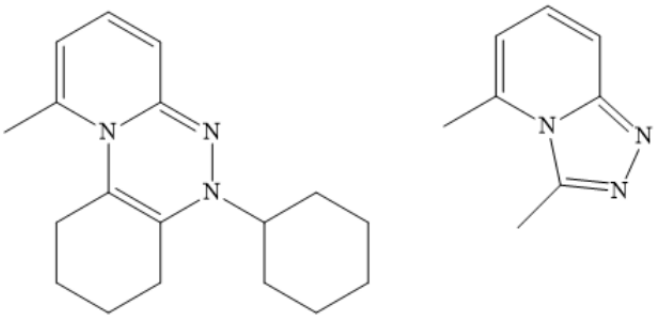

Fig 5: By-products from Fischer indole synthesis

5. Fischer synthesis is most useful for generating 2,3-substituted indoles, where the indole formation can be done in a single pot because it is not required to separate the intermediate aryl hydrazones. However, in the Fischer synthesis, unsymmetric ketones give two region-isomeric 2,3-displaced indoles with a region-selectivity depending on medium acidity, hydrazine substitution and steric effects. 1,2-diketones can give both mono-indoles and bis-indoles, which are usually formed by strong acid catalysts in refluxing alcohols.

The next big breakthrough in indole synthesis came from the development in organometallic chemistry in the second half of the 20th century, as advances in theoretical chemistry allowed us to understand the reactions of transition metals better.

The Fischer indole synthesis is ultimately a purely classical organic synthesis, as it involves techniques such as electrophilic substitution for a π-electron-rich system. Similar approaches were used to develop alternate ways of synthesizing indole, for example, the Leimgruber-Batcho indole synthesis (Fig 6) [6], which uses orthonitro toluenes as the starting product instead. A likewise formation of enamine using a base, is followed up by cyclisation, in this case, using Raney nickel as a reducing agent. This technique became more used than the Fischer indole synthesis in certain pharmaceutical applications [6], because of higher yield in some cases, and better availability of starting products. Yet, the reaction did not prove to be as versatile as latter developments.

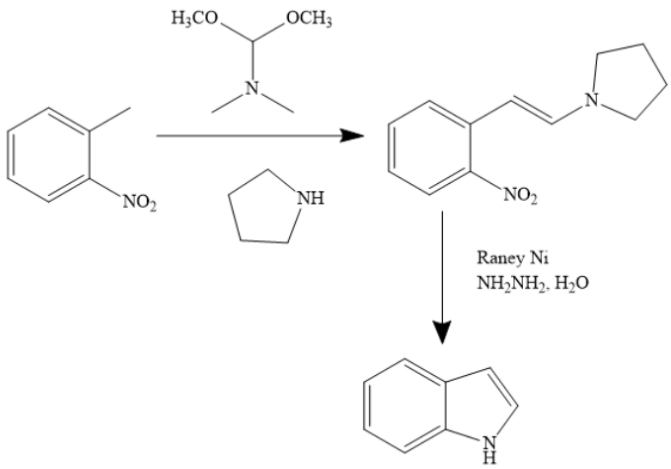

Fig 6: Leimgruber-Batcho indole synthesis

In the middle 20th century, with the refinement of transition metal chemistry, it was learnt that for most organic functional groups, there exist some transition metal, which will produce a reactive organometallic intermediate. This complex formation is specific and eliminates the need for protecting groups (which form a temporary protection of a different reactive functional group, and is removed later), as the selective activation of one functional group is possible.[7]

Prior Work Done in Palladium Chemistry

Palladium exists in two major oxidation states – Pd0 and PdII , apart from the normal Pd0 metal .Palladium is also a good catalyst because it has a relatively low activation barrier between the oxidation states of 0 and +2 corresponding to the stable d10 and d8 configurations. These oxidation states are important for oxidative insertion and reductive elimination. The lower the activation barrier of the transition from 0 to +2 and back again, the more rapidly the catalytic reactions can take place. The faster and more favourable these reactions are, the higher the turnover rate for the catalyst.

Palladium and some other transition metals are suitable at holding on to reactants. Transition metals to the left end, such as titanium, are very reactive, and have a tendency of oxidizing and then not reacting further. At the other extreme, metals like gold are too less reactive for the organic catalytic syntheses palladium is used for. Palladium will hold on to reactants, let them

rearrange into products, and then allow them to leave/ desorb after the reaction is done.

Palladium can be oxidized to form palladium salts. In particular, [PdCl2]n is a bridged polymer, which is itself insoluble in organic solvents, but readily forms soluble solvates when reacted with a metallic chloride or an alkyl nitrile.[7]

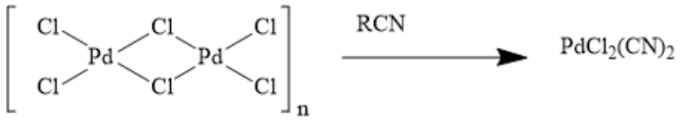

Fig 7: Formation of Palladium Salts

Palladium salts were observed to be highly electrophilic, e.g., towards alkene double bonds. The organometallic intermediate was flexible in synthesis of many different organic functional groups (Fig 8).

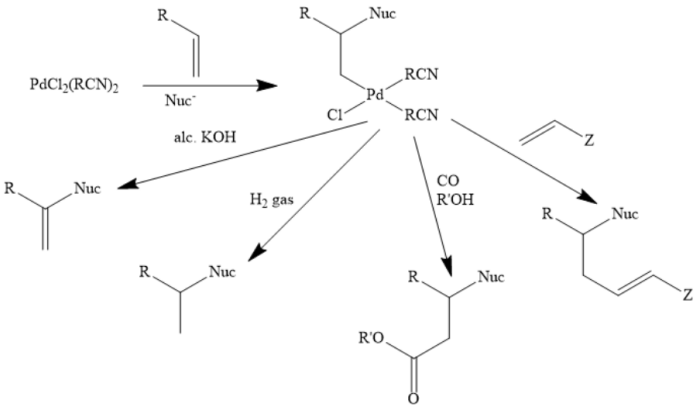

Fig 8: Initial Usage of Palladium Salts in Organic Synthesis

This was the start of the usage of palladium and techniques in organometallic chemistry in heterocyclic synthesis[8]. Soon, researchers in Japan, with Itihara being a prominent name, was able to synthesize more complex multicyclic aromatic compounds from less complex multicyclic compounds [9] (Fig 9). Here, the role of palladium diacetate as a catalyst was to add onto two benzene rings as an electrophilic aromatic substituent, forming a bridge in its +2 oxidation state, and then coupling together the rings.

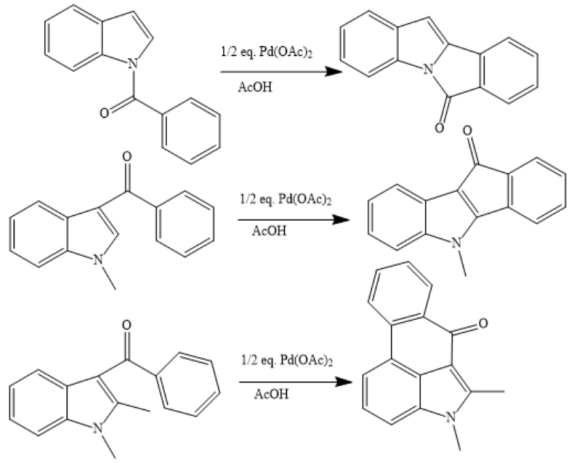

Fig 9: Using $Pd(OAc)_2$ to form complex multicyclic aromatic compounds

The versatility of palladium salt-catalyzed heterocyclic synthesis was further found when it was used to insert substituents in an indole nucleus [9] (Fig 10) and perform a single-electron-transfer to form dihydroindole compounds[10](Fig 11).

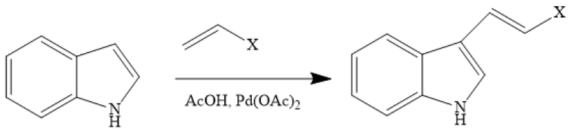

Fig 10: Insertion of Substituent in Indole nucleus

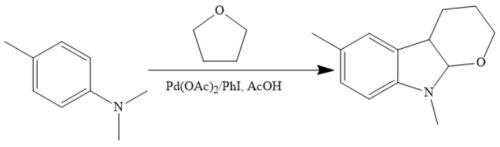

Fig 11: Formation of Dihydroindole compounds using $Pd(OAc)_2$ catalyst

This salt of palladium was applied later in the Larock indole synthesis.

Use of Alkynes in Organometallic Synthesis

The growth in organometallic chemistry also led to the development of synthesis techniques using alkynes. For example, several functional groups were successfully synthesized from alkynes, using transition metals as catalysts, including the synthesis of pyridines (Fig 12) and quinones (Fig 13)[11], among others.

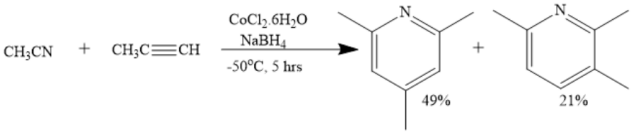

Fig 12: Transition Metal Catalyzed Alkyne Synthesis of Pyridines

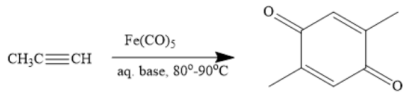

Fig 13: Transition Metal Catalyzed Alkyne Synthesis of Quinones

Using palladium catalysts in particular, alkynes were shown to undergo an intermolecular cocyclotrimerization[11] to form aromatic molecules. In these reactions, two molecules of alkynes reacted with one other molecule to form the benzene nucleus (Fig 14). Excess of alkynes are used with the Palladium-ring complex being the limiting reagent.

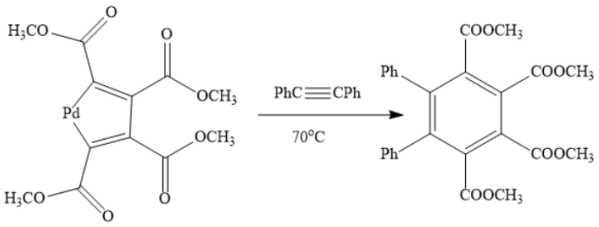

Fig 14: Palladium Catalyzed Organic Synthesis Using Alkynes

This success inspired chemists to try annulating onto an existing aromatic benzene ring to form multicyclic compounds, using alkynes and a palladium catalyst. However, intermolecular attempts to annulate onto alkynes using a palladium catalyst on an existing aromatic nucleus resulted in either multiple alkyne insertion or subsequent cyclization back onto the initial aromatic nucleus[18] (Fig 15).

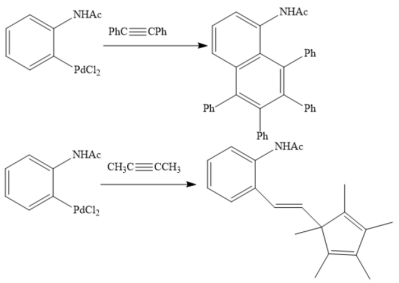

Fig 15: Multiple Alkyne Insertion Products in Palladium Catalyzed Annulation Attempts

Now, the main Larock indole synthesis is discussed.

Reaction Discussion:

Initial Discovery

Richard Larock, a professor of chemistry at Iowa State University, had palladium-catalyzed organic synthesis, as one of his primary interests. Prior to the discovery of the Larock indole synthesis, his research group had done extensive work on annulation (ring formation) using dienes. His research group was able to carboannulate 1,2-dienes using ortho-substituted aryl halides using palladium catalysts[12] (Fig 16).

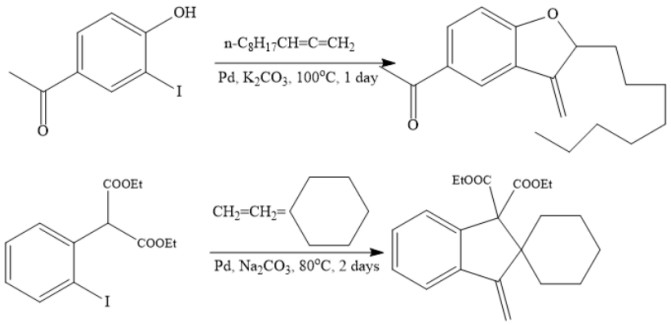

Fig 16: Carboannulation of 1,2 dienes

This prior success inspired the group into using alkynes in annulation reactions.

In 1991, Larock reported a flexible high-yield method of synthesizing indole and indole-family compounds using palladium acetate salt as the catalyst. Ortho-iodoaniline and its derivatives were used as the starting material.[1] This was the initial discovery of the Larock indole synthesis. (Fig 17a) An example is shown below. (Fig 17b)

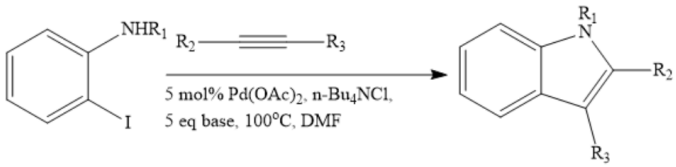

Fig 17a: Initial Larock Indole Synthesis

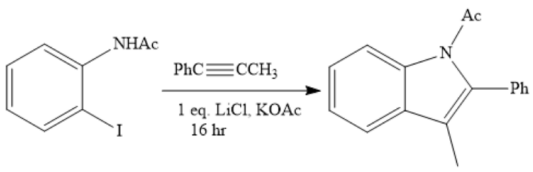

Fig 17b: Example of a Larock indole synthesis

This reaction was a significant improvement upon

previous techniques for multiple reasons.

1. Firstly, compared to earlier techniques such as the Fischer indole synthesis and the Leimgruber-Batcho indole synthesis, the Larock indole synthesis was more flexible. The three variable chains (marked R1, R2, R3) could be a diverse set of organic groups, and this enabled a greater variety of compounds to be synthesized.
2. The yield obtained was much better than the low yield attempts to synthesize indoles from N-methyl-p-toluidines and acetylenedicarboxylate esters, as done by Sakakibara and Tanaka.[13] (Fig 18) Although the earlier techniques used were similar, earlier methods produced many difficult-to-separate by-products too.
3. In many cases, the Larock indole synthesis is regioselective, enabling us to selectively synthesize in drug design applications.

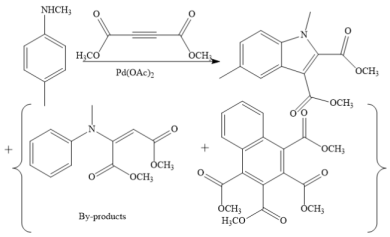

Fig 18: Sakakibara's Low-Yield Indole Synthesis using Alkynes with Palladium Catalysts

Potassium carbonate and acetate bases proved to suitable bases for the Larock indole synthesis. A catalytic amount of PPh3 was used initially, but later it was found out that Ph3P was superfluous and did not affect the reaction activity that much. Moreover, using PPh3 at temperatures higher than 100o C often caused multiple alkyne insertion.

Initially, n-Bu4NCl was used as the base by Larock's research team, using only 1 equivalent of it per equivalent of iodoaniline. However, later the researchers discovered that using lithium chloride (LiCl) was more effective and reproducible.[1] It was further observed that LiCl has a critical concentration, as using more than 1 equivalent of lithium chloride lowered yield due to multiple insertion products as by-products, the same problem Sakakibara faced.

Larock later published a much-more detailed stoichiometric analysis[14] of the Larock indole synthesis in 1998. There, Larock investigated the stereochemical and stoichiometric aspects of the reaction, as well as key aspects – the effect of concentration of alkyne used, a comparison of various bases used at different temperatures, and the stereochemical effect of various substituents on the alkyne. These experiments enabled Larock to determine the reaction mechanism.

Reaction Mechanism and Method of Determination

Prior reactions using palladium catalysts, where palladium formed a bridge between the aryl nucleus and the functionality that is being added[9], allowed Larock to deduce the possible reaction mechanism of the Larock indole synthesis. (Fig 19)

The steps in this reaction are:

1. Reduction of the Pd(OAc)2 to Pd0. Both Pd2+ and Pd0 states have space for two more ligands, and in the solution, palladium is usually bound to solvent molecules or other ions.
2. Coordination of the chloride ion from the base to form a chloride-ligated Pd0 species[14]. The chloride ion comes from the base (LiCl or n-Bu4NCl).
3. Oxidative addition of the aryl iodide onto the chloride-ligated Pd0 species.
4. Coordination of the alkyne onto the palladium atom of the resulting arylpalladium intermediate.
5. Subsequent Regioselective syn-insertion into the arylpalladium bond of the alkyne. If the alkyne is substituted, due to this step, the substituents in the final indole molecule are always cis with respect to the double bond. The regioselectivity will be elaborated on later.
6. Nitrogen displacement of the halide in the resulting vinylic palladium intermediate. This forms a six-member heteroatom-containing palladacycle.[14]
7. Reductive elimination of the indole molecule to regenerate the Pd0 species that was formed.

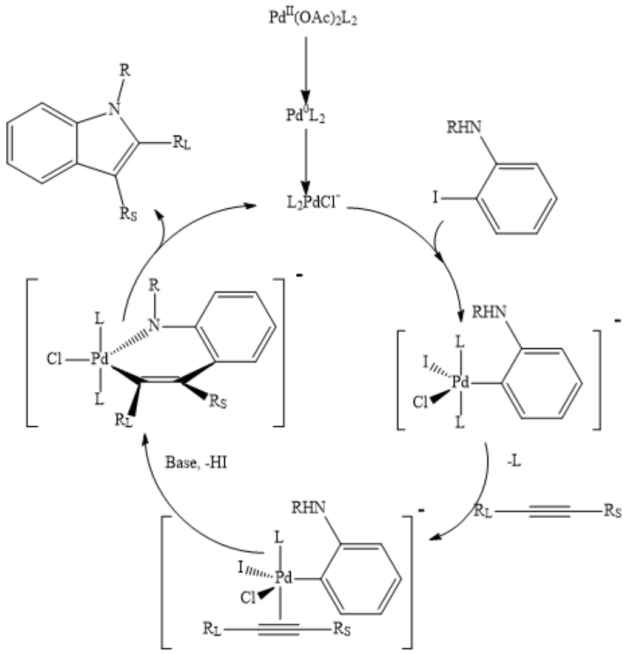

Fig 19: Reaction Mechanism of the Larock Indole Synthesis

Here, RL denotes a sterically larger substituent, while RS denotes a sterically smaller substituent.

Among the various steps, the redox reaction thus described had been encountered in earlier palladium-catalyzed synthesis reactions.[10] The chloride ligation step was theorized by Amatore[17] earlier to explain various kinetic results associated with the oxidative addition of aryl iodides to Pd0 species. The syn-addition of alkynes had been theorized earlier as well in particular the low-yield method of obtaining indole described earlier.[13] The fact that the palladium catalyst is regenerated means that we can recycle this reagent, leading to better efficiency in the synthesis procedure.

The palladacycle formed is too short-lived to isolate it in the Larock indole synthesis, however in a study by Driver and Hartwig[22], an arylpalladium amide analogously formed a carbon-nitrogen bond, providing more support to the reaction mechanism described.

It was observed that in place of ortho-iodoanilines, ortho-bromoanilines cannot be used as the starting material. This is because the insertion of palladium in the aryl-bromide bond, and subsequent elimination of HBr is not thermodynamically favourable (HI is a stronger acid, more ionic, and exits faster).

The work by Pfeffer et al[18] regarding chemical kinetics for various halides in palladium-catalyzed reactions hypothesized that the formation of the carbon-nitrogen bond for only a single alkyne insertion, as in the Larock indole synthesis, is due to the poor σ-donor properties of the iodine atom in the reactant. That is why, usage of ortho-chloroanilines causes the formation of multiple insertion products instead.

Concentration of Alkynes Used

It was observed that higher yields were obtained with higher concentration of alkynes, especially for volatile alkynes.[1] (Fig 20). With 5 equivalents of alkynes, Larock obtained an 80% yield. However, even with lower concentrations of alkynes, yield was significant. The higher yield for more concentration of alkynes meant that in practical uses, an excess of alkynes is used (especially if product purity is important).

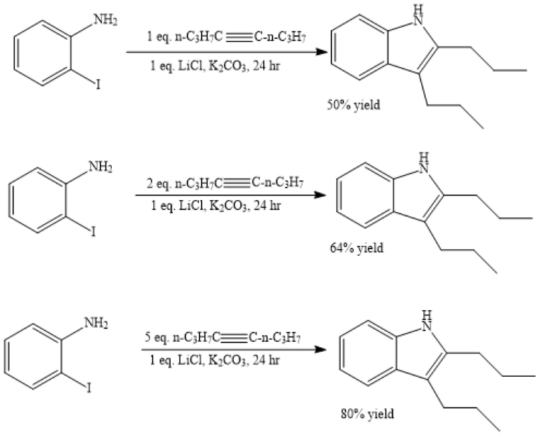

Fig 20: Effect of Concentration of Alkyne on Yield (Palladium catalyst used)

Kinetic Comparison of Various Bases Used

1. **KOAc**: Potassium acetate has a low yield if neither LiCl nor PPh3 is used. If 1 equivalent of LiCl is used, the yield is good if performed at a temperature of 120o C. LiBr and LiI cannot be used in place of LiCl if KOAc is used due to kinetic effects. There was no reaction if the temperature was lowered to 100o C or lower. At the temperature of 120o C, addition of PPh3 was unnecessary as it gave a lower yield.
2. **K2CO3**: Potassium carbonate has slightly lower yield than even potassium acetate if LiCl is not added. However, potassium carbonate is compatible with both LiBr and LiI, and gives an

ever higher yield if slightly more concentration of LiCl is used. There is similar behavior with respect to temperature at 120o C, but K2CO3 can even produce indoles at 100o C.

3. **NaOAc**: Produces an inferior yield than the equivalent potassium base.
4. **Na2CO3**: Produces an inferior yield than the equivalent potassium base.

Thus, potassium acetate and potassium carbonate are the ideal bases for the Larock indole synthesis.

Effects of Substituents on the Nitrogen of the Iodoaniline

The Larock indole synthesis was flexible enough that the nitrogen atom in the final indole nucleus could have a variety of substituents. Larock was successful in using N-methyl-2-iodoaniline, 2-iodoacetanilide and N-tosyl-2-iodoaniline (Fig 21) as starting products. This was especially significant because previous work by Pfeffer[18] with different reaction conditions was not able to undergo high-yield annulation if 2-iodoacetanilide was the starting product. The reactivity increases if electron withdrawing substituents are present in the benzene nucleus, because of the stabilized intermediate. The intermediate has a negative charge, so electron withdrawing substituents lower the transition state energy.

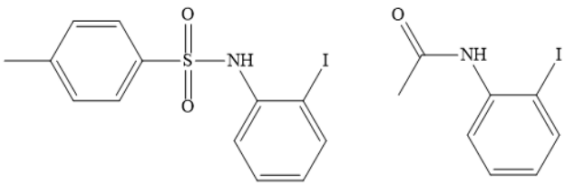

Fig 21: N-tosyl-2-iodoaniline (left), 2-iodoacetanilide (right)

Effect of Substituents in the Alkyne

The alkyne used in the Larock indole synthesis can have a wide variety of substituents, without affecting the yield. Simple unhindered alkyl-substituted alkynes, such as 4-octyne, also provide a high yield, unlike previous attempts[18]. However, the reactivity for such unhindered alkynes tends to be high, and if the temperature is too high, there is possibility of multiple insertion. Thus, moderately sterically hindered alkynes, for example, tertiary butyl substituted alkynes or trimethylsilyl alkynes, tend to give a higher yield in such cases, due to lack of multiple insertion by-products.

There is thus an interacting trade-off between maximizing yield with unhindered alkynes at moderate temperatures versus hindered alkenes at higher temperatures.

The alkyne can also have other functional groups such as alcohols. Indeed, analogous work in hydroarylation of alkynols by Cacchi[19][20] showed that alkynols can be utilized in the conditions used in the Larock indole synthesis. (Fig 22)

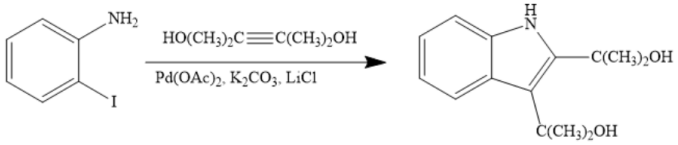

Fig 22: Indole synthesis using Hydroxyalkyl-substituted Alkynes

Regioselectivity

It was confirmed by works by both Larock[14] and Cacchi[20] that the reaction for an asymmetric alkyne is highly regioselective. The sterically bulky group ends up in the 2-position in the indole nucleus formed, nearer the nitrogen. Only the preferred isomer is formed if the alkyne is bulky, e.g., 1-phenyl-1-propyne. If the alkyne is less bulky, e.g., 2-pentyne, both isomers are formed, with the preferred isomer highly dominating. [1](Fig 23)

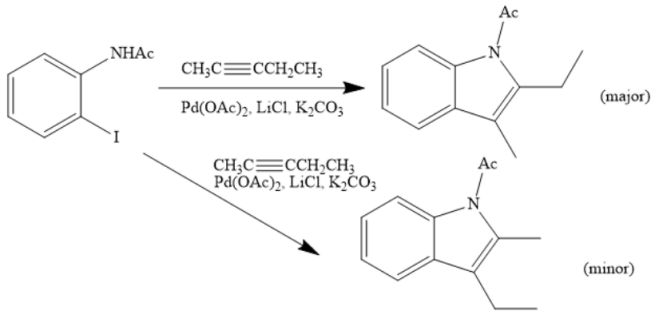

Fig 23: Major and Minor products for Asymmetric Alkynes

The transition state (Fig 24) places the more sterically hindered group next to palladium. (Fig 24) The controlling factor behind this regioselective behavior

is likely the steric hindrance present in the developing carbon-carbon bond between the benzene ring and the alkyne. Thus, the orientation of the alkyne prior to the syn-insertion into the aryl-palladium bond is in a way such that the steric strain in the developing carbon-carbon bond.[14] Here, it is important that the alkyne is parallel and cis-coordinated to the arylpalladium bond for the syn addition to take place.

A similar regioselectivity is observed for annulation using alkynes onto an aromatic ketone using manganese catalysts, as in the works of Liebeskind et al.[21]

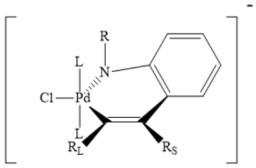

Fig 24: Transition State having the Larger Substituent nearer the Palladium Atom

In addition to the steric effects, if one of the substituents in an alkyne contains a hydroxy (-OH) group, it occupies the position nearer the palladium atom (RL in Fig 24), and thus is the substituent on the 3-position in the finally formed indole, because of coordination between the lone pair of oxygen and the palladium atom. This is analogous to the similar effect in hydroarylation of alkynes.[19][20]

Limitations due to Unexpected Products

Some reactants do not form the standard product formed in the Larock indole synthesis. These exceptional behaviors are mentioned now:

1. Migration of Acetyl Group from N-acetyl-iodoaniline

   If the alkyne has one substituent containing an alcohol group, the final product has the alcohol acylated instead with the previously acylated amine forming a free amine instead. This is due to a base-catalyzed acetyl group shift.[14] (Fig 25)

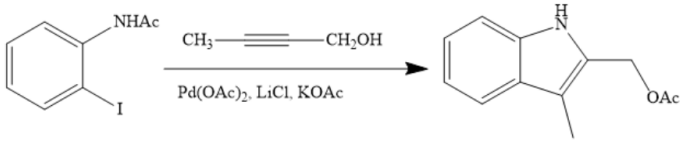

Fig 25: Shift in Acetyl Group in Larock Indole Synthesis

2. Reaction of Hindered Disilyl alkynes to form Monosilyl substituted indoles

   If a hindered disilyl alkyne is used as the starting reactant, for example, bis(trimethylsilyl)acetylene, the product formed by the conditions for Larock indole synthesis has only one trimethylsilyl group. The elimination of one silyl group is likely due to steric factors. (Fig 26)

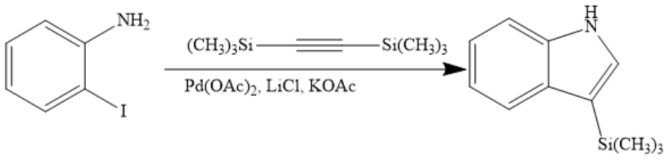

Fig 26: Bis(Trimethylsilyl)acetylene forming trimethylsilylindole

Other Limitations

1. Cost-Effectiveness due to Availability of Reagents

   Ortho-iodoaniline is far better reactive than ortho-bromoaniline and ortho-chloroaniline. However, ortho-iodoaniline is less easily available in the laboratory and is costlier. Thus, the Fischer indole synthesis remains a good alternative technique for those indole molecules which can be synthesized without the problems of the Fischer indole synthesis. However, if complex salts of palladium are used, ortho-bromoaniline is also reactive.

2. Limitation if 7-Position has Bulky Substituent

   If the 7-position in the indole nucleus has a bulky substituent, it is difficult to use the Larock indole synthesis due to steric hindrance when the palladacycle is formed.

Immediate Impact in Other Synthesis Techniques

It was recognized by Larock that this reaction mechanism enabled a broad variety of synthesis. For example, if one of the substituents on the alkyne was a silyl group (such as Si(CH3)3), the silylindole produced could further form a variety of substituted indoles through disilylation and halogenation (Fig 27), followed by the Heck reaction to form more complex substituted indoles.[15](Fig 28)

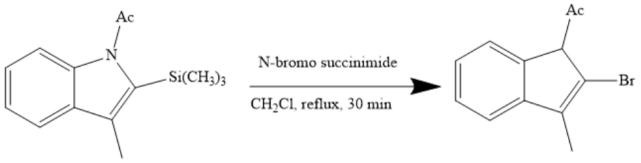

Fig 27: Halogenation of Silylindole formed to form acylated 2-bromindole

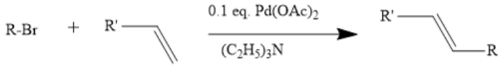

Fig 28: Heck reaction

Further Annulation Reactions

The success in synthesizing indole led to Larock successfully reporting the synthesis of a wide variety of heterocyclic compounds based on palladium-catalyzed addition of alkynes. These included 1,2-dihydroisoquinolines (Fig 29), benzofurans, benzopyrans and isocoumarins.[16] The reaction conditions used were similar – this included 5% molar Pd(OAc)2, sodium or potassium acetate or carbonate as the base, and LiCl or n-Bu4NCl as the base, with DMF as the solvent.

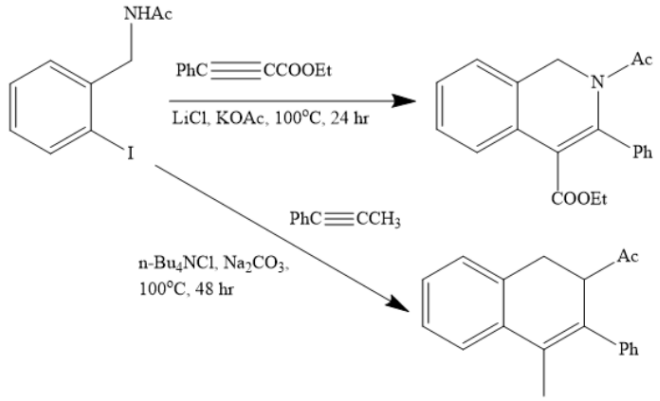

Fig 29: Synthesis of 1,2-dihydroisoquinolines

The synthesis of dihydroisoquinolines was slow with ortho-iodobenzylamine as the starting product, with a low yield. However, using the corresponding acetamide produced a much better yield[16], as shown in Fig 29.

Analogous annulation reactions can also be done using oxygen as the nucleophilic atom. However, the analogous reaction with ortho-iodophenol instead of ortho-iodoaniline was extremely slow and only possible for selective alkynes at a higher temperature.

Unlike what Larock had observed for the indole synthesis, where less hindered alkynes also reacted very fast, only heavily hindered alkynes (such as trimethylsilyl alkynes) reacted with ortho-iodophenol. These reactions were also regioselective, like the Larock indole synthesis. Fig 30 shows the synthesis of a benzofuran, while Fig 31 shows the synthesis of an isocoumarin in this manner.

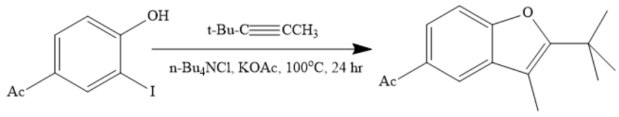

Fig 30: Synthesis of Benzofuran using analogous reaction conditions (palladium catalyst)

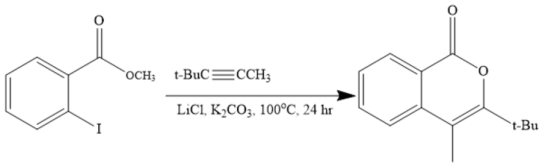

Fig 31: Synthesis of Isocoumarin using analogous reaction conditions (palladium catalyst)

If the alkyne used is asymmetric, these analogous reactions are generally regioselective, with the bulkier substituent appearing near the heteroatom (nitrogen or oxygen). The reason is the same as that mentioned for the regioselective nature of the Larock indole synthesis.[16]

Just as the Larock indole synthesis can be used to further prepare more substituted indole compounds, the analogous reactions for oxygen as the heteroatom can be used to prepare more substituted benzofurans. For example, reacting a triisopropylsilyl alkyne produces a triisopropylsilyl substituted benzofuran, which is readily desilylated to form 3-substituted benzofurans, which would be difficult to produce directly (as the alkyne would not have been hindered enough for the direct reaction). CH3CN is often used as a solvent for that reaction.(Fig 32)

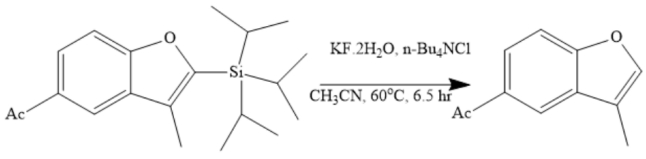

Fig 32: Using Triisopropylsilyl Benzofuran to form 3-substituted Benzofurans

Applications in Synthesis:

Here, some synthetic applications of the Larock indole synthesis are elaborated upon. Indole being such a biologically active molecule, has been used to develop many families of alkaloids.

Alkaloid Synthesis using Intermolecular Larock Synthesis of 3,4 Fused Tricyclic Indoles

The indole nucleus can be found in a variety of natural biomolecules. Some of the most important biologically active indole family molecules are 3,4-fused indoles, including lysergic acid and Penitrem A. Some of the amides of lysergic acid, called lysergamides, have agonist or antagonist activity at various serotonin and dopamine receptors. Penitrem A (tremortin), on the other hand, is an indole-diterpenoid mycotoxin, that can affect the central nervous system by bypassing the blood-brain barrier.

These molecules are synthetically challenging due to its tricyclic nature. Some of them are shown below. (Fig 33) Previous attempts to synthesize similar 3,4-fused tricyclic indoles usually involved introduction of functional groups in the 3- and 4- positions of an already existing indole and then cyclization.[23] However, electrophilic aromatic substitution prefers the 5- and 7-positions.Thus, using the Larock indole synthesis, one can determine an alternate pathway, where the alkyne is inserted as a functional substitutent onto the 4- position of an ortho-iodoaniline. A sample reaction is given below (Fig 34).

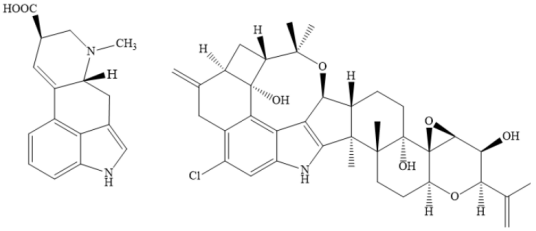

Fig 33: 3,4-Fused Tricyclic Indoles: Lysergic Acid (left), Penitrem A (Right)

Such techniques can even be used to generate macrocyclic indole compounds with 18 compounds in a ring.[26](Fig 34b)

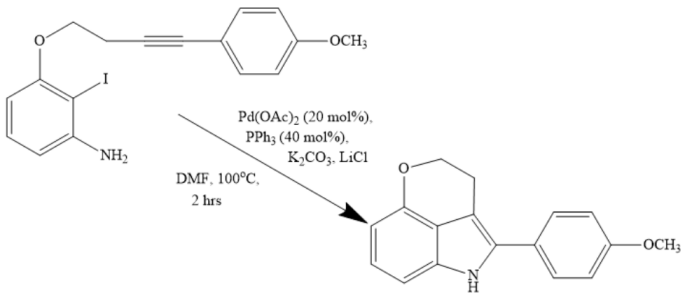

Fig 34a: Intramolecular Larock Synthesis of 3,4-Fused Tricyclic Indoles

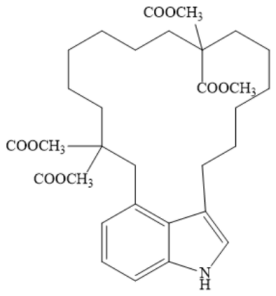

Fig 34b: 18-cyclic macrocyclic indole prepared

A small amount of palladium catalyst is then sufficient for Larock indole synthesis. This technique was used by Shan et al[23] to generate the alkaloid Fargesine, extracted from the root and stem of Evodia fargesii. The insertion of the alkyne chain was done by imine formation from an aldehyde and subsequent reduction. (Fig 35a) Likewise techniques can be done to synthesize complex 3,4-fused alkaloids by choosing appropriate starting materials. Using a tosylated 3,4-fused indole compound can be further used to synthesize tetrahydropyrroloquinoline (Fig 35b)[26].

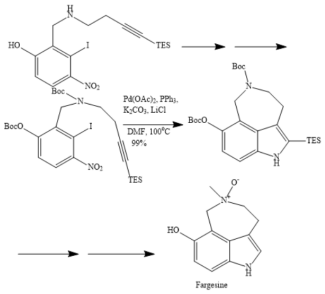

Fig 35a: Synthesis of Fargesine

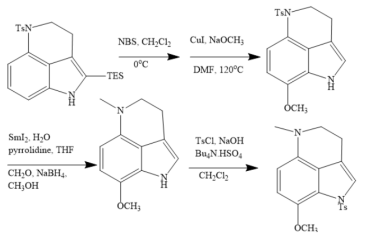

Fig 35b: Synthesis of Tetrahydropyrroloquinoline

Tetrahydropyrroloquinoline can be used as an antifungal and cytotoxic agent.

Use in Synthesis of HIV Protease Inhibitors

Palladium catalyzed annulation of alkynes, e.g., acetylenes, has also been used in medicine. For example,

an important class of antiviral drugs used against HIV/AIDS are HIV protease inhibitors, which prevent viral replication by selective binding to HIV-1 protease, a retroviral aspartyl protease virus essential in the HIV life-cycle. One of those candidates is L-754394 (Fig 36)[24]. Such drugs need furopyridine structural units to be synthesized, which can be manufactured using the Larock indole synthesis-inspired heterocyclic annulation using alkynes with palladium catalysts.

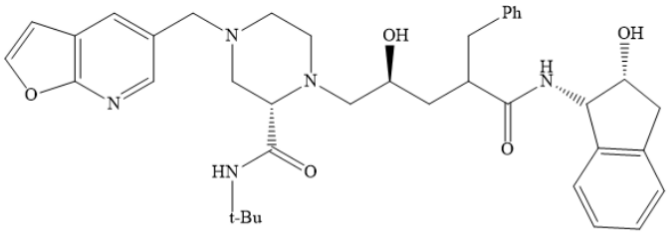

Fig 36: L-754394

The furopyridine nucleus can be manufactured using palladium-catalyzed coupling ethyl 2-hydroxy nicotinate, and the following synthesis is possible. (Fig 37) Here, kinetic studies showed that n-BuNH2 worked as a better base than KOAc or K2CO3. The concentration of alkynes is typically around 5 equivalents at least. CuI is used alongside palladium as a catalyst for higher yield. In large-scale production, CuI is more cost-effective.[24] The starting benzene nucleus has an electron-withdrawing ester group, because electron-rich indole formation is unstable.

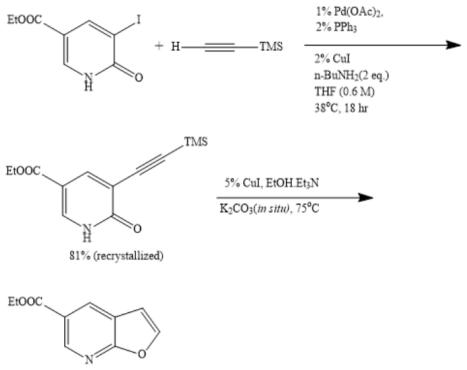

Fig 37: Synthesis of Furopyridine using Palladium-Catalyzed Alkyne Insertion

Manufacture of Serotonin Family Anti-Migraine Drugs with Heterocycle Moiety

The monoamine serotonin is a neurotransmitter with multifaceted functions, including regulation of mood, cognition, reward, learning and memory. Its role as a vasoconstrictor in the brain makes it beneficial in migraine therapy. However, its potential as a pharmaceutical agent is limited due to in vivo rapid metabolism.[25] N, N-dialkyltryptamines have been developed as 5-HT1D receptor agonists for the treatment of migraines. This include sumatriptan. Synthesis of analogous MK-0462 compound cannot be done using the Fischer indole synthesis, due to presence of benzyl triazole moiety – the Fischer synthesis is sensitive to additional functional groups such as the triazole, which would lead to a very poor yield. Such compounds need to be synthesized using techniques such as the Larock indole synthesis, which are not sensitive to triazole and other nitrogen-containing heterocycle substituents. Fig 38a shows the compounds serotonin, sumatriptan and MK-0462. Fig 38b shows the Larock synthesis of this drugs. Sumatriptan can also be synthesized in this manner.[25]

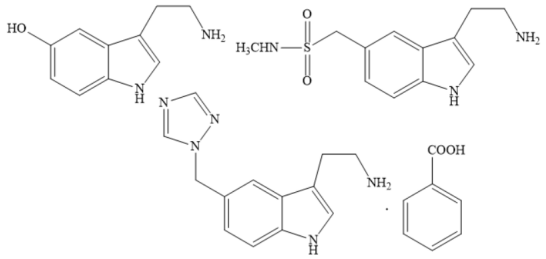

Fig 38a: Serotonin (Top-left), Sumatriptan (Top-right), MK-0462 (Bottom)

The alcohol used in this Larock reaction must have a protecting group initially, and then be deprotected for the reaction to have high yield of the product desired.

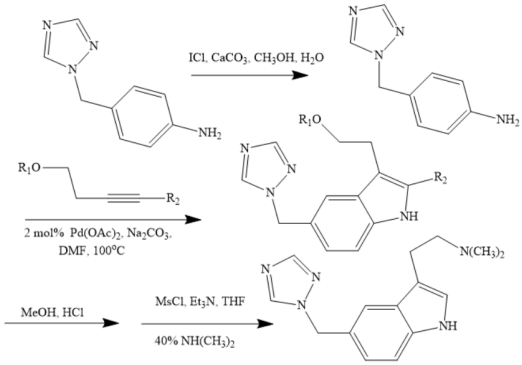

Fig 38b: Synthesis of Serotonin-Family Migraine Drugs using Larock indole synthesis

Use in Total Synthesis of Complex Macromolecules: Chloropeptin II (Complestatin)

Chloropeptin II (Complestatin) is a complex macromolecule isolated from Streptomyces lavendulae, noted for its HIV infectivity and cytopathic effects. It

is structurally similar to glycopeptide antibiotics like vancomycin, but notably, one of the characteristic biaryl ether linkages is replaced with a biaryl linkage. The compound is atropisomerically chiral.

Chloropeptin II was discovered to have HIV inhibitory and cytopathic effects. Biogenetically, both chloropeptin I and chloropeptin II are linear non-ribosomal peptides that have undergone oxidative phenolic coupling to produce a rigid cross-linked architecture.[41]

Boger et al[27] used an intramolecular Larock indole synthesis to create the initial macrocyclization. The overall retrosynthetic broken bonds are indicated along with the structure in Fig 39, while the Larock indole synthesis used is provided in Fig 40. Later, a different synthesis by Wang et al[41] used intramolecular Suzuki–Miyaura and SNAr reactions for the construction of two macrocycles in Chloropeptin II by the formation of aryl–aryl and aryl–aryl ether bonds, respectively.

Complex salt catalysts which allow ortho-bromoaniline to be used are used in the process described by Boger et al[27]. The intramolecular Larock indole synthesis guarantees that the indole cyclization is regioselective. [27]

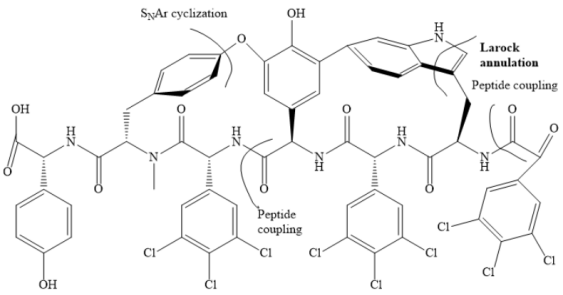

Fig 39: Retrosynthetic breakdown of bonds in total synthesis of Chloropeptin II

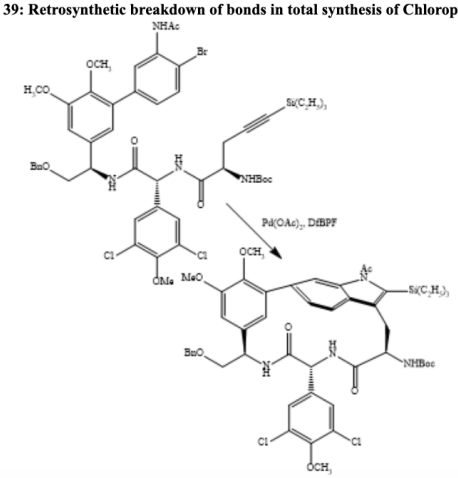

Fig 40: Larock annulation step in total synthesis of Chloropeptin II

Use in Total Synthesis of Chemotherapy Targets

Dictyodendrins are a family of alkaloids having a pyrrolo[2,3-c] carbazole moiety and having at least one sulphate group. They are isolated from the sponge of Dictyodendrilla verongiformis. These compounds are telomerase enzyme inhibitors. Telomerase enzymes are overexpressed in tumor cells compared to normal cells, thus, dictyodendrins are possible targets for cancer chemotherapy.[28]

The total synthesis of dictyodendrins involve a palladium-catalyzed Larock indole synthesis which then undergoes N-alkylation, bromination, followed by Buchwald-Hartwig amination, to form an intermediary which undergoes a carbazole formation to give dictyodendrins. [28]The Larock indole synthesis step is given in Fig 41a, while the final product is shown in Fig 41b. In this case, t-BuONa was experimentally determined as the best base, with DMSO at 160o C being the best solvent.

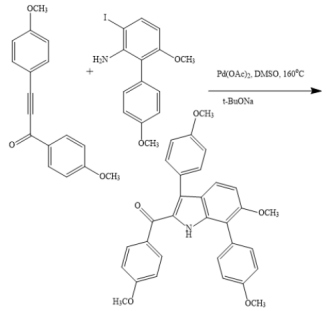

Fig 41a: Larock indole synthesis in dictyodendrin synthesis

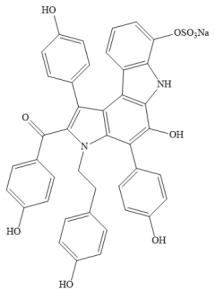

Fig 41b: Dictyodendrin B

Use in Antibacterial, Antiviral and Antifungal Alkaloid Synthesis: Total synthesis of Dragmacidin

Dragmacidin D is a member of the Dragmacidin family of heterocyclic bis(indole) compounds. It has one stereogenic centre and was found to be a potent inhibitor of serine/threonine phosphatases. It is known to have antiviral, antibacterial, and antifungal activity, as well as invitro cytotoxicity against murine leukemia, and some varieties of human lung, colon and mammary cancers.[29]

The structure of Dragmacidin D consists a central pyrazinone core with two indole substituents, one of which is further linked to an aminoimidazole unit. The first indole nucleus is created through the Larock

indole synthesis, while the second indole nucleus (separately prepared) then attaches in a Friedel-Crafts type arylation. The catalyst used here is a more complex chelated sandwich-compound of palladium. n-Bu4NBr was used as the base here. Fig 42a shows the Larock indole synthesis step in this synthesis[29] while Fig 42b shows the final product.

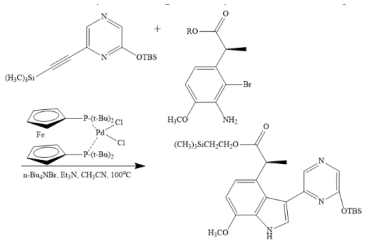

Fig 42a: Larock indole synthesis in Dragmacin D synthesis

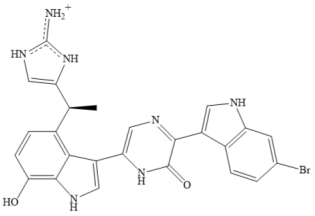

Fig 42b: Dragmacin D

Subsequent Development and Conclusion:

Here, the subsequent developments in the Larock indole synthesis and subsequent development in palladium-catalyzed annulation reactions are covered, especially those involving alkynes. Many of these developments were crucial in the synthetic methodologies developed, that were covered in the past section.

Effect on Regioselectivity in Larock indole synthesis with 2-alkynylpyridines

If the alkyne is asymmetric, Larock[1][14] had earlier provided a steric hindrance based explanation to explain the regioselectivity in the indole formed regarding position of substituents. The sterically bulky group ends up in the 2-position, near the nitrogen atom. In 2008, Senanayake et al[30] published their findings where the regioselectivity seemed to be affected if a 2-alkynylpyridine is used, i.e., one of the substituents in the alkyne is a pyridine ring, attached at the 2-position (ortho to the nitrogen atom). In particular, he compared the molar ratio of isomers formed when the alkyne used is 2-cyclopentylethynylpyridine versus cyclopentylethynylbenzene (compounds shown in Fig 43).

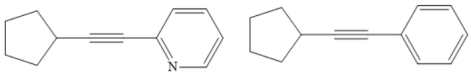

Fig 43: Compound A (2-cyclopentylethynylpyridine) (left), Compound B (cyclopentylethynylbenzene) (right)

For Compound A, the ratio of isomers was unusually high (94:6). For Compound B, the ratio of isomers was 67:33, despite the two compounds having similar sizes sterically. If 3-cyclopentylethynylpyridine was used, the ratio was 68:32, similar to that for Compound B. This is because if one of the substituents are such that there is possible σ-donation from an electron-rich centre (e.g., nitrogen atom in the 2-cyclopentylethynylpyridine) to the palladium atom, regioselectivity is increased.[30] Thus, 2-pyridin-2-ylindoles are preferred over 3-pyridin-2-ylindoles. The modified reaction mechanism is given in Fig 44.

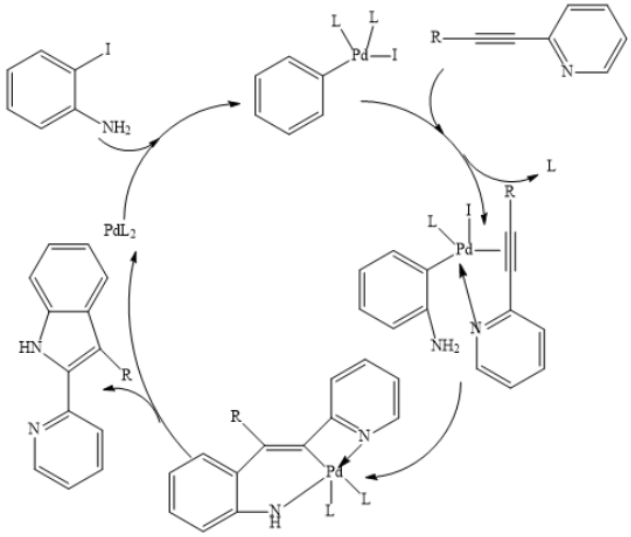

Fig 44: Modified Reaction Mechanism for 2-alkynylpyridines

The difference in this reaction mechanism is that the nitrogen atom forms an additional coordinate bond with the palladium atom to form one additional palladacycle. Thus, the regioselectivity is enhanced because of this additional ring formation.[30]

Development of Alternate Catalysts and Reacting Conditions

The Larock indole synthesis and the analogous heterocyclic annulation reactions both involve an expensive palladium catalyst, which is not cost-efficient for large-scale industrial use. Moreover, the original reaction conditions given by Larock involved specialized ancillary ligands, high temperatures, and an excess of the alkyne.

Ni(dppp)Cl2, or 1,3-bis(diphenylphosphino) propanonickel chloride is a nickel-based catalyst that was used by Weng, Xie et al earlier [31] to form isoquinolines starting from 2-haloaldimines and alkynes under very mild conditions. This catalyst proved to be useful for the Larock-type indole synthesis, as well as the synthesis of ketones of isoquinolines from ortho-iodobenzamides. 2 equivalents of Et3N needs to be used as the base, and CH3CN is the ideal solvent for this reaction. The reaction is shown in Fig 45.

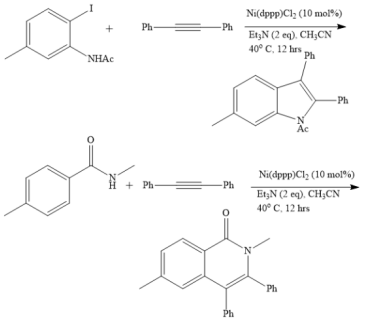

Fig 45: Nickel-synthesized heteroannulation of ortho-iodoacetanilides (top), ortho-iodobenzamides (bottom)

Weng et al further determined that this reaction does not involve any aryl radical intermediate, and instead consists of Ni(0) formed from in situ reduction of the complex salt, as the catalyst, which then undergoes oxidative addition into the C-I bond. The deprotonation of the amide hydrogen by the Et3N base forms a five-membered nickelacycle intermediate. Then there is a coordinative insertion of alkynes, followed by reductive elimination to obtain the product.[31]

This reaction mechanism is strikingly like the original Larock indole synthesis.

Monguchi et al also developed a more effective synthetic method for indole derivatives that did not need the LiCl salt. The catalysts used were palladium on carbon (Pd/C) and NaOAc in heated NMP (N-methyl-2-pyrrolidone).[33]

He, Du, Liu et al developed an N-heterocyclic carbene based palladium complex for the Larock indole synthesis. This catalyst proved to be not only suitable for ortho-iodoanilines, but also suitable for ortho-bromoanilines. Use of complex salts of palladium became a better catalyst for cost-effectiveness, as ortho-bromoaniline is cheaper and easier available in labs than ortho-iodoaniline [32]. A common catalyst used is Ferrocenyl NHC-Pd-Pyridine complex, shown in Fig 46.

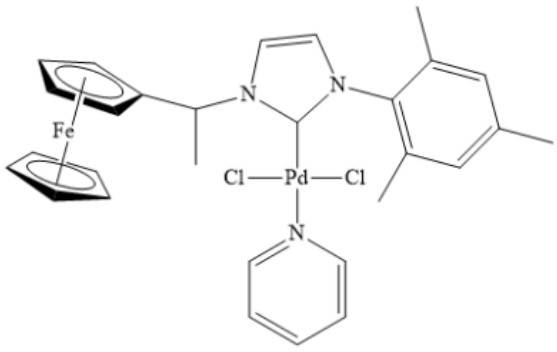

Fig 46: Ferrocenyl NHC-Pd-Pyridine Complex

This catalyst takes part in a similar mechanism as the Larock indole synthesis. The PdII atom reduces to Pd0 in situ from the complex and gets oxidatively added to the C-X bond to produce a PdII-aryl complex. Then the alkyne coordinates and inserts into the Pd(II)-aryl bond. Deprotonation, followed by reductive elimination forms the final product.[32]

So far, most procedures have used soluble catalysts, including soluble palladium catalysts with costly phosphine ligands. Separation of the catalytic material from the reaction mixture, and ligand contamination of the reaction mixture cannot be allowed for biological applications. Thus, heterogenous catalysts are preferred for such applications.[34]. Earlier, Batail, Bendjeriou, et al, had used the heterogenous palladium catalyst Pd/C or Pd/NaY for indole and benzofuran syntheses under both ligand-free and salt-free conditions.[35]

However, that technique lacked recyclability of the catalyst due to relative lack of catalyst activity. Later, Batail, Bendjeriou, et al derived a hybrid catalyst based on SBA-15 mesoporous silica modified by grafted palladium complexes bearing other phosphine or cyano ligands.[34] The covalent immobilization of Pd+2 complexes allowed for a highly ordered mesostructured. This heterogenous catalyst was particularly useful for multiple recycling, as the material could be used to convert ortho-iodoaniline into indole over multiple cycles without significant dip in reactivity.[34] Recycling of the expensive palladium-based reagent made the synthesis procedures much cheaper.

Catalyst Loading using Nanochemistry Techniques

From an industrial point of view, reducing catalyst loading is highly desirable and thus transition-metal nanoclusters (NCs) are attractive for chemical and medical research. Onishi, Oikawa et al[36] prepared N, N-dimethylformamide (DMF) stabilized transition-metal nanoparticles, which showed high catalytic capacity in other organometallic reactions such as the Ullmann reaction and the Suzuki coupling reaction. Those DMF-stabilized palladium nanoparticles were also suitable as catalysts for the Larock indole reaction. The NCs were recycled at least 3 times, and the typical particle size of the nanoparticles is between 1-3 nm.[35]

Further Development in Synthetic Techniques

1. Buchwald Modification

The Larock indole synthesis inspired newer methodologies for the Fischer indole synthesis. In particular, Buchwald et al [37] discovered a palladium-catalyzed method for the preparation of indoles via the Fischer indole synthesis, using the same catalyst used in the Larock indole synthesis. This proved to be extremely useful especially if the starting material was a hydrazone. Such starting materials are not stable enough to undergo the traditional Fischer indole synthesis. The base used in this reaction (shown in Fig 47) is usually bulky, such as t-BuONa.[37]

The use of a Pd/BINAP-based catalyst produced N-aryl benzophenone hydrazones in good yields. After this, the N-aryl benzophenone hydrazones can be converted to indole products via the in-situ Fischer cyclization. This method can also be used to generate N-alkylindoles via N-aryl-N-alkylbenzophenone hydrazones. [37]

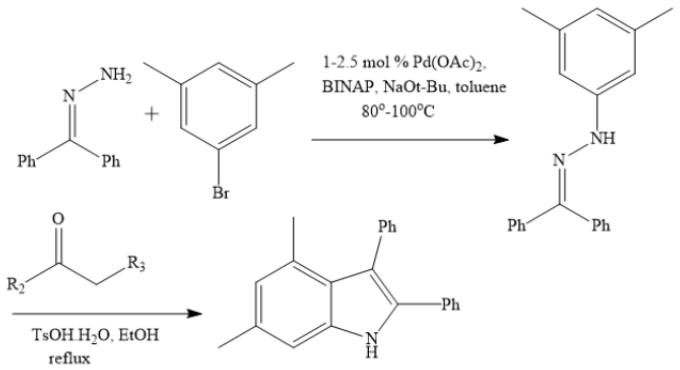

Fig 47: Palladium Catalyzed Fischer Indole Synthesis

2. Palladium-catalyzed Oxidative Indole Synthesis

The palladium catalyst was found to be useful in oxidative cyclization of N-aryl imines into indole as well. (Fig 48) Wei, Deb et al[38] observed that this oxidation can happen even in presence of aerobic oxygen and is highly tolerant of other functional groups. Two-fold oxidative cyclizations also happen. An imine-enamine transformation, followed by a Larock-like reaction, where a six-membered palladacycle is formed, ended by reductive elimination, is the mechanism here as well.

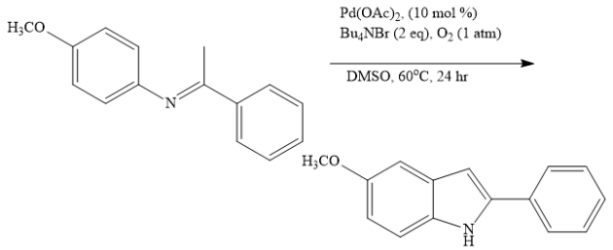

Fig 48: Palladium-catalyzed Aerobic oxidation of Imines to Indoles

Versatility when paired with Sonogashira Coupling

The Larock indole synthesis becomes very versatile when paired with Sonogashira coupling, yet another innovative technique in organometallic chemistry.[8] [39] Using a palladium catalyst as well as a copper cocatalyst, it forms a carbon-carbon bond between a terminal alkyne and an aryl or vinyl halide. (Fig 49)

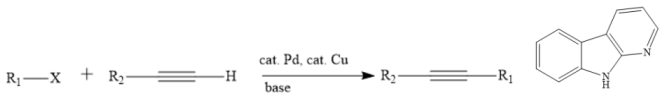

Fig 49: Sonogashira coupling

Fig 50: beta-Carboline

The reason why the Larock indole synthesis becomes even more powerful when used alongside Sonogashira coupling is because they can be together used to synthesize large polycyclic alkaloid macromolecules. The typical procedure goes this way:

a. Formation of aryl halide and Sonogashira coupling with alkyne to form alkyne substituent
b. Larock indole synthesis of the substrate with ortho-iodoaniline

Such steps have been used by Pan and Bannister[40] to successfully synthesize beta-carboline (norharmane) (Fig 50) containing alkaloids.

Fig 51 shows the likewise synthesis of indolopyridocoline triflate, a beta-carboline containing alkaloid, whose synthesis involves preparing the substrate for the Larock indole synthesis using Sonogashira coupling. Fig 52 likewise shows you the synthesis step of the alkaloid mitragynine that involves the Larock indole synthesis (the substrate comes from Sonogashira coupling).[40]

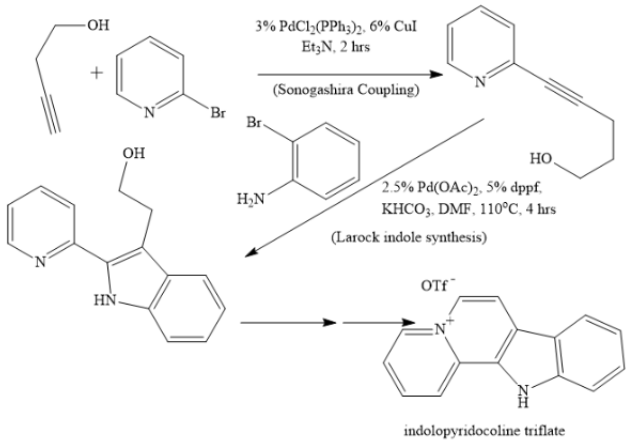

Fig 51: Synthesis of Indolopyridocoline Triflate

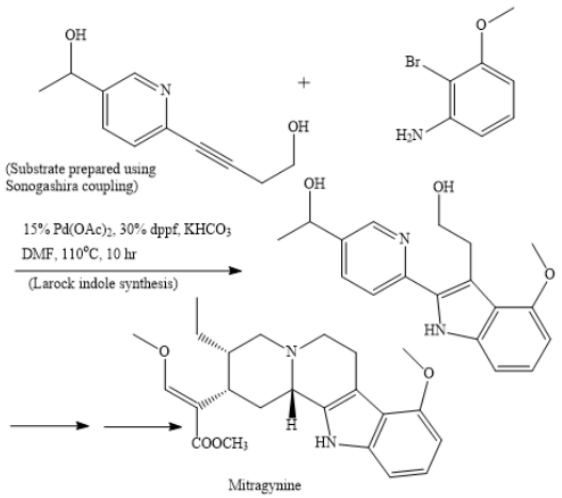

Fig 52: Synthesis of Mitragynine (Larock indole synthesis step)

(Fig 53a-53b). These drugs have been manufactured using techniques not involving the Larock indole synthesis or other palladium/transition-metal catalyzed heteroannulation. These drugs are manufactured purely using classical techniques mostly. With the innovation in cheap heterogenous catalysts [34], is it possible to synthesize these drugs using the latest organometallic techniques?

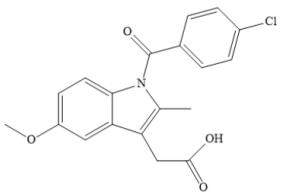

Fig 53a: Indometacin

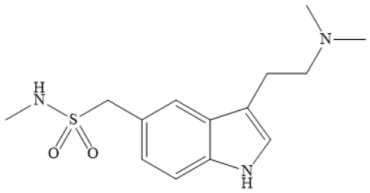

Fig 53b: Triptan (Sumatriptan)

Limitations

1. The effects of solvents and bases regarding the Larock indole synthesis have not been fully formulated. Depending on the compounds used as reactants, chemists are still dependent on experimentally using various combinations of bases and solvents and choosing the one with the highest yield. Thus, the choice of reaction conditions is only empirically determined and is a theoretical black-box.
2. Although multiple synthesis techniques to make indole have been discovered, there is no one synthesis technique is the one-size-fits-all best method for all indole family molecules. Depending on the target molecule, purity level, and cost-effectiveness, and functional groups, different reaction techniques are best-suited. An organic synthesist should be aware of the various methodologies possible in this regard.
3. Palladium is not the only metal that is suitable as the catalyst. Nickel catalysts have been tried for the Larock-like indole synthesis. It is yet to be determined if other transition metals, possibly cheaper, could be used as catalysts in any way or not.

Future Suggestions

1. The indole molecule is the nucleus of a variety of important drugs, such as indometacin and triptan
2. Further studies should be undertaken, using tools from inorganic chemistry, to determine why certain transition metals act as good catalysts for these reactions, while others do not. It should also be studied why certain ligands bound to transition metals enable the synthesis to occur, while others do not. The kinetic effects of various neighboring ligands are not fully understood yet and need to be pursued.

Conclusion:

Overall, the Larock indole synthesis is a cornerstone in modern organometallic synthetic techniques. The technique shows remarkable flexibility compared to past techniques and has seen applications in numerous fields. It has been used in synthesis of alkaloids, HIV protease inhibitors, anti-migraine drugs, chemotherapy targets, antibacterial chemicals and antifungals, and total synthesis of macromolecules, as well as development of better catalysts. It is a method that is likely to come to use to an organic synthesist in some way or the other. Recent innovations in other areas of chemistry, such as solid-state chemistry and nanochemistry, are pushing the frontiers of organometallic chemistry forward, and allowing us to develop newer novel methodologies.

Citations

1. Larock, Richard C.; Yum, Eul Kgun; *J. Am. Chem. Soc*. **1991**, 113, 6689-6690
2. Fischer, E.; Hess, O. *Chem. Ber.* **1884,** 17 (1), 559–568
3. Borsche, W. Justus Liebigs Ann. *Chem*. **1908**, 359 (1-2), 49-80
4. Robinson, B. *Chem. Rev.* **1963**, 63, 4, 373-401
5. Robinson, B, *Chem. Rev.* **1969**, 69, 2, 227-250
6. Maehr, H.; Smallheer, J.; *J. Org. Chem*. **1981**, 46, 8, 1752–1755
7. Hegedus, Louis S., Angew. *Chem*., **1988**, 27 (9), 1113-1226
8. Tsuji. J, Palladium in Organic Synthesis, ISBN: 1436-6002, 148-179
9. Itahara, T; J. *Org. Chem*. **1985**, 50, 25, 5272-5275
10. Sakakibara, T., Karasumaru, et al, *J. Am. Chem. Soc*., **1985**, 107, 22, 6417-6419
11. Schore, Neil. E.; *J. Am. Chem. Soc*.; **1979**, 101, 24, 7410-7412
12. Larock, Richard C.; Berrios-Pena F., Colleen A.; *J. Org. Chem*. **1991**, 56, 8, 2615-2617
13. Sakakibara, T.; Tanaka. Y.; Yamasaki, S.; *Chem. Let*., **1986**, 15 (5), 797-800
14. Larock, Richard C.; Yum. E. K.; Refvik. M. D.; *J. Org. Chem*. **1998**, 63, 7652-7662
15. Beletskaya I.; Cheprakov A.V.; *Chem. Rev.* **2000**, 100, 8, 3009-3066
16. Larock, Richard C.; Yum. Eul K.; Doty. M.J.; *J. Org. Chem*. **1995**, 60, 3270-3271
17. Amatore, C.; Azzabi. M.; *J. Am. Chem. Soc*., **1991**, 113, 22, 8375-8384
18. Maassarani. F.; Pfeffer. M.; Le Borgne. G.; *J. Chem. Soc*., **1987**, 8, 565-567
19. Cacchi. S.; *Pure Appl. Chem*., **1990**, 62, 4, 713-722
20. Cacchi. S,; *Pure Appl. Chem*., **1996**, 68, 1, 45-52
21. Liebeskind. L.S.; Gasdaka J.R.; McCallum. J.S.; *J. Org. Chem*., **1989**, 54, 3, 669-677
22. Driver. M.S.; Hartwig J.F.; *J. Am. Chem. Soc*., **1997**, 119, 35, 8232-8245
23. Shan. D.; Gao. Y.; Jia. Y.; *Angew. Chem*. **2013**, 52, 4902-4905
24. Houpis. I.; Choi. W. B.; Reider. P.J.; *Tetra. Let*., **1994**, 35, 50, 9355-9358
25. Chen, C.-Y, Lieberman, D.R., Larsen, R.D., *Tetra. Let*., **1994**, 35, 38, 6981-6984
26. Gao. Y.; Shan. Y.; Jia. Y.; *Tetra. Let*., **2014**, 70, 5136-5141
27. Boger. D.; Garfunkle. J.; Kimball. S.; *J. Am. Chem. Soc*. **2009**, 131, 16036-16038
28. Liang. J.; Hu. W.; Tao. P.; Jia. Y.; *J. Org. Chem*. **2013**, 78, 5810-5815
29. Jackson. J.; Kobayashi. H.; Steffens. S.; *Angew. Chem*. **2015**, 54, 9971-9975
30. Senanayake. C.; Roschangar. F.; Liu. J.; *Tetra. Let*., **2008**, 49, 363-366
31. Weng. W.; Xie. J.; Zhang. B.; *Org. Biomol. Chem*., **2018**, 16, 3983-3988
32. He. P.; Du. Y.; Liu. G.; Pang. G.; *RSC Adv.,* **2013**, 3, 18345-18350
33. Monguchi. Y.; Mori. S.; Aoyagi. S.; *Org. Biomol. Chem*., **2010**, 8, 3338-3342
34. Batail. N.; Bendjeriou. A.; Djakovitch. L.; *Appl. Catal A. Gen*. **2010**, 388, 179-187
35. Batail. N.; Bendjeriou. A.; Djakovitch. L.; *Adv. Synth. Catal*. **2009**, 351, 2055-2062
36. Onishi. K.; Oikawa. K.; Yano. H.; *RSC Adv*., **2018**, 8, 11324-11329
37. Wagaw. S.; Yang. B.; Buchwald. S.; *J. Am. Chem. Soc*.; **1999**, 121, 44, 10251-10263
38. Wei. Y,; Deb. I.; Yoshikai. N.; *J. Am. Chem. Soc.; 2012*, **134**, 9098-9101
39. Sonogashira. K.; *J. Organomet. Chem*., **2002**, 653, 46-49
40. Pan. X.; Bannister. T.; *Org. Lett*.; **2014**, 16, 6124-6127
41. Wang, Z.; Bois-Chussy, M., Jia, Y., Zhu, J.; Angew.

Chem. **2010**, 49, 11, 2018-2022

Comparing experiences with mental health issues to perceptions of their prevalence: A study of Duke University undergraduates

Sean P. Woytowitz

Volume 1, Issue 2

Article Synopsis

Although much attention has recently been brought to the rapidly growing issue of college student mental illness, an analysis of the correlates, prevalence, and perceptions of mental illnesses in college has yet to be conducted. To close this gap in the literature, this study examined Duke University undergraduate students' experiences with the symptoms of the most common disorders of depression and anxiety. The data indicated an alarmingly high prevalence of these disorders, demographic groups that may benefit from mental healthcare outreach, as well as students' overestimation of the prevalence of anxiety on campus. These findings can inform shifts in the approach of mental healthcare for college students and can guide future inquiries into experiences with mental illness at other American universities.

Graphic by Cindy Ju

Comparing experiences with mental health issues to perceptions of their prevalence: A study of Duke University undergraduates

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https://doi.org/10.55894/dv1.23

Abstract

Mental illness affects all populations but has disproportionately affected college students in the recent past. Universities provide a unique setting in which students often live away from their families and must make an effort to stay socially engaged. While plenty of research on mental health has been conducted on children and adults, the field is lacking in research on college-aged individuals. The present study identifies the frequency of symptoms of depression and anxiety among the Duke University undergraduate community, as well as demographic factors that are correlated with these symptoms. The data were obtained through a survey asking students about their experiences with well-established symptoms of Major Depressive Disorder and Generalized Anxiety Disorder. The data indicate that students at Duke University experience symptoms of depression and anxiety at a higher rate compared to the average 18-29 year old person in the United States. Other findings include a greater prevalence of mental illness symptoms among low-income students, students with small social-circles, and non-religious students. The results of this study and related studies to follow will be used to inform improvements to mental health counseling and outreach to at-risk individuals at universities across the United States.

Introduction

Mental illness, though always prevalent in our society, has experienced recent growth in the number of diagnoses and individuals receiving treatment (U.S. Department of Health and Human Services, 2020). While this is potentially attributable to the expansion of mental healthcare and opportunities for diagnosis, the severity of the issue remains apparent. Also, the number of people being medicated for undiagnosed depressive and anxious disorders is at a concerningly high level (Whitaker, 2005). The greatest prevalence of Major Depressive Disorder is found among the adult population aged 18-25, where nearly one in every six individuals has experienced a depressive episode in the past year (U.S. Department of Health and Human Services, 2020). These young adults are often facing a multitude of conflicts for the first time as they begin to be pulled in a variety of directions by their social circles, familial attachments, academic preoccupations, financial needs, and their personal desires.

The feeling of isolation that may occur when given the task to independently make critical, long-term decisions (i.e. career choices) for the first time is an experience shared by many adolescents in present day society, and particularly in the United States given its expectation of self-sufficiency surrounding adulthood. This term, "isolation," is one that can be found repeatedly in literature on depression and anxiety, both academic and recreational. It may also contribute to the success of mental health initiatives that push a dialogue centered around the idea that "You are not

alone" (Ford-Paz et al., 2020). It is also abundantly clear that social isolation, whether self-imposed or caused by other factors, contributes to the development of depressive symptoms, suicide attempts, and low self-esteem in adolescents (Hall-Lande et al., 2007). As well, the recent COVID-19 pandemic has only fueled an increase in the stress and sadness associated with mental and physical isolation (Thakur & Jain, 2020). The isolation that many young adults who experience depression and anxiety go through may mean that their mental illnesses are not readily apparent to onlookers. As such, it is plausible that the prevalence of these illnesses may be underestimated. The present research into this relationship– experiences with mental illness symptoms and perceptions of the prevalence of such symptoms– could therefore contribute to a greater "You are not alone" dialogue and could inform numerous adjustments to mental illness interventions at the community level.

The DSM-5: Depression and Anxiety

To analyze the nature of depression and anxiety in young adults and properly interpret perceptions of these illnesses, it is necessary that we understand how healthcare professionals and leaders in the field of psychology view these illnesses. The most common form of depression, known as Major Depressive Disorder (MDD) in the DSM-5, is what most individuals think of when they hear the term "depression;" an intense state of sadness or general loss of interest. According to the DSM-5, an MDD diagnosis requires five or more of the following symptoms during the same two-week (or longer) period that are a change from previous functioning: depressed mood, loss of pleasure, weight loss or gain, hypersomnia or insomnia, psychomotor agitation or retardation, fatigue, feelings of worthlessness or guilt, decreased concentration, and thoughts of death or suicide. At least one of the symptoms must be a depressed mood or loss of pleasure (American Psychiatric Association, 2013).

Additionally, when an individual is considered anxious in numerous contexts at a clinically significant level, this is most closely associated with what the DSM-5 refers to as Generalized Anxiety Disorder (GAD). A GAD diagnosis requires excessive anxiety and worry that is difficult to control and occurs more days than not for at least 6 months about several events. It also requires three of the following symptoms to be present: restlessness, fatigue, difficulty concentrating, irritability, muscle tension, and sleep disturbances (American Psychiatric Association, 2013).

It should be noted that for both MDD and GAD, the DSM-5 requires that the symptoms cause clinically significant distress, cannot be attributed to the effects of a substance or to another medical condition, and cannot be better explained by an alternative disorder. Additionally, MDD requires a lack of manic or hypomanic episodes (as these may be characteristic of Bipolar Disorder as opposed to MDD). In the DSM-5, MDD and GAD are placed into separate categories (depressive and anxious disorders, respectively) (American Psychiatric Association, 2013). However, the rate of comorbidity between these disorders remains significant with estimates as high as 80% (U.S. Department of Health and Human Services, 2020).

Mental Illness in Young Adults

In developed countries, an increasing amount of attention is currently being given to the topic of mental illness. With rates of diagnoses increasing, this expansion of awareness is a necessity and should continue to grow in order to achieve destigmatization and a common understanding of these disorders (Schomerus et al., 2012). The amplification of social media and online and cellular communication has been shown to have negative impacts on the mental health of young adults, specifically on both anxious and depressive symptoms (Thomée et al., 2007). Seeing as how these platforms will only continue to develop, more care should be taken in how communities perceive mental health moving forward (Thomée et al., 2007).

Additionally, a study analyzing severe mental illness (SMI) in a broad sample of adults found that young adults were about three times as likely to have been treated for a SMI when compared to older adults, and their prevalence for such disorders was higher (Karlin et al., 2008). While exact mechanisms behind these discrepancies between generations have not been identified, the contributing factors likely include shifts in social norms, the use of technology, higher emotionality in young adults, and a potentially high rate of underdiagnosis in older adults (Casey et al., 2010). Most individuals are also not taught mental health literacy or skills in resilience when they are in school, resulting in a wide distribution of perceptions

of mental health, its prevalence, and its stigmas (Kelly et al., 2007). This may even contribute to the variations that are seen between genders and cultures in methods of mental health disclosure and willingness to participate in conversations on mental health (An & McDermott, 2014). Beyond this, it appears that there has been minimal change over time in attitudes towards individuals with mental illnesses in spite of the fact that mental health literacy is slowly increasing as more attention is brought to the issue (Schomerus et al., 2012). Ultimately, the summation of this evidence suggests that mental illness needs to be addressed at a greater depth, particularly in the case of young adults, through channels such as mental health education and mental healthcare outreach.

College campuses can provide researchers with in-depth insight into the mental health of young adults. This particular community has a variety of shared potential mental illness triggers, including but not limited to tuition-related financial instability, the pull from social media to be one's best self at all times, the often overwhelming force of rigorous academics on one's psyche, and the separation from one's family given the often high proportion of students living on their own. Previous inquiries into college students' mental health have been conducted but many have failed to consider the impact of college culture on the development of mental illnesses. One online study identified that male students, off-campus students, and younger students were less inclined to pursue treatment for mental health issues (Yorgason et al., 2008). However, these trends may depend on the institutional structure of the university, the size of the university, the importance of stable mental health to each university's student body, the gender norms integrated into the culture of each university, and countless other factors that are dependent on the environment in which these students find themselves (Wang & Degol, 2016). Some colleges are able to better conduct outreach to students in need of assistance or to advertise the availability of their counseling services, therefore improving the relationship that the students have with mental health. Accordingly, conducting inquiries into student mental illness is best done at the individual university level, as these subtle and complex cultural trends can be identified.

While identifying the intricacies in each individual college environment may be a rather daunting and tedious task, it is not one that should be taken lightly or delayed. Researchers identified that nearly half of college-aged individuals had experienced a mental disorder in the past year (Blanco et al., 2008). They also found that college students were more likely to develop substance use disorders than non-college-attending persons of the same age, yet college students were less likely to have diagnoses for such disorders (Blanco et al., 2008). The immense prevalence of mental health issues in young adults is a significant problem on its own, but the addition of the stress induced by a college environment appears to only worsen these illnesses. These results become even more concerning when one considers Blanco's finding (2008) that fewer than 25% of mentally ill survey participants had sought treatment in the past year, indicating that a large majority of college students with mental disorders are suffering in silence. Providing these students with tailored changes to their school's counseling services should be a priority for mental health researchers, mental healthcare providers, and university administrations.

The Present Study

This study will focus on the relationship between experiences with depressive and anxious symptoms and perceptions of their prevalence within the Duke University undergraduate community. This student body was selected given the unique nature of the young adult experience at a top university. By looking at the relationship between previous experiences with MDD and GAD during college and estimates of how prevalent these disorders are amongst the Duke student body in particular, trends can be more easily seen and common experiences can be inferred between participants. Additionally, this study will examine how certain demographic factors correlate with mental illness symptom experiences and particular perceptions of their prevalence. These data may also reveal trends within the Duke University community that are not readily apparent.

Considering the increasing prevalence of mental illness and the variety of college-specific stressors, it is hypothesized that Duke undergraduates will underestimate the prevalence of mental illness symptoms as reported by the study's participants. Similarly, we expect that the prevalence of mental

illness symptoms among Duke students will be greater than that of the similarly aged American population. We also predict that, given the results of Hall-Lande et al.'s study, students with lower involvement in school activities and social groups will report more experiences with the DSM-5's mental illness symptoms (2007).

It is expected that the results of this study will have implications for the ways in which Duke's mental health programs approach treatment for students and the ways in which Duke University conducts outreach to students in need. For example, if students pursuing a particular field of study at Duke University are found to experience mental health issues at a significantly greater rate, then Duke's Counseling and Psychological Services (CAPS) can focus on advertising its services and providing outreach to students within this academic department. Through such mechanisms, this study may help alleviate the burden of mental illness that has been placed on college students. Additionally, the expectation is that similar studies may be conducted in other communities and on other college campuses to reveal relationships between their demographics, mental illness prevalence, and mental illness perceptions. Ultimately, these data could be compared and synthesized to determine any commonly present trends between these variables for young adults in the academic environment.

This study will have true social significance, as studies such as Schomerus's meta-analysis, mentioned above, imply that current methods of raising awareness of mental illnesses as biological conditions that require treatment have not shifted attitudes towards mental illness in general (2012). While this perspective is important and valid, this study takes a novel approach in an ultimate attempt to shift attitudes regarding mental health; it will use the relationship between prevalence and perceptions as the driving argument as to why these conditions are significant and why they require attention. Not only will this study potentially reveal contributing factors to mental illness in the Duke undergraduate community, but by aiding a particular community in seeing the extent to which they have experienced symptoms of mental illness, it will potentially strengthen the treatment process and could lead to a positive shift in sensibilities regarding mental health.

Methods

Participants

As stated above, the present study sampled students completing their undergraduate studies at Duke University. In total, 269 individuals completed the survey; this sample consisted of 98 men, 168 women, and 3 non-binary individuals. Additionally, the distribution of class year in the sample was 107 first year students, 105 second year students, 36 third year students, and 21 fourth year students. All academic fields of study were represented in the sample except for foreign languages, and all racial groups were represented except for Native Hawai'ian/Pacific Islander. There were no criteria for which participants' data were eliminated from the study, apart from failure to complete the survey. Students were recruited using flyers and Duke-associated Facebook groups, as well as Duke's Sona System which provides psychology students with course credit for participation in research.

The Experience and Perception Survey

The survey was designed to both preserve the anonymity of participants while still collecting enough personal information to analyze demographic correlates of mental illness experiences and perceptions. The survey was conducted through the Qualtrics software, was pre-approved by Duke's Institutional Review Board, and contained five blocks:

Block 1: Consent

This block informed students that the survey they were completing would ask them for personal information, and that it would deal with potentially triggering topics such as depression. Participants checked a box to provide their consent.

Block 2: Demographics

This block asked participants for their undergraduate year (Freshman, Sophomore, Junior, or Senior), their gender identity (male, female, non-binary, or prefer not to say), their area of study, their race, their estimated family income (separated into categories by the most recently established American tax brackets), the size of their social networks (small or large), the number of Duke extracurriculars they are involved in, and their level of religiosity.

Block 3: Experience with Mental Illness Symptoms - Depression

This block began with a preface that asked participants to answer the questions honestly and in terms of their entire college career (not at the particular

moment that the survey was completed). The questions in this block asked participants about their experiences with the symptoms for MDD as listed in the DSM-5. The block finished with a question regarding the number of symptoms experienced within a particular time frame to gauge whether or not the participant likely met the full DSM-5 criteria for MDD, and a question regarding any previous treatment for depression or depressive symptoms.

Block 4: Experience with Mental Illness Symptoms - Anxiety

This block once again began with a preface that asked participants to answer the questions honestly and in terms of their entire college career, not that particular moment. The questions in this block asked participants about their experiences with the symptoms for GAD as listed in the DSM-5. The block finished with a question regarding the number of symptoms experienced within a particular time frame to gauge whether or not the participant likely met the full DSM-5 criteria for GAD, and a question regarding any previous treatment for anxiety or anxious symptoms.

Block 5: Perceptions of Mental Illness Symptoms Among Duke Students

In the fifth block, students were asked to approximate what percentage of Duke students experienced different numbers of depressive and anxious symptoms (i.e. 0-2 of the symptoms, 3-4…). They were also asked to approximate what percentage of Duke students met the criteria for clinical anxiety or depression in college and what percentage have been treated for clinical anxiety or depression. This block finished by asking participants if they could think of any potential causes of their mental illness symptoms (for example, stress brought on by COVID-19) or any programs that Duke could have implemented to help them with their symptoms.

The survey ended with a message thanking the students for participating, providing participants with an email for any future inquiries regarding the study and its results, and providing participants with a link to Duke's mental health resources. It should be noted that MDD and GAD DSM-5 criteria were used to ensure the validity of the mental illness measures, to ensure the collection of substantial data given how common these disorders are, and to most accurately reflect the struggles that young adult students may be dealing with. Also, the Qualtrics software was designed to safeguard against any repeat or fraudulent responses, and to delete any data that was incomplete to allow participants to safely withdraw from the study at any time.

Data Analysis

The data obtained through the Qualtrics software was analyzed in CSV format through Microsoft Excel. Independent-samples t-tests were used to compare the frequency of mental disorders among each of the demographic groups (such as males and females). In cases where there were more than two demographic groups present, t-tests were used to compare each group to the one that was designated as the "control". The results of the statistical tests were also plotted using Microsoft Excel. All data were ultimately erased from the devices on which the analyses were conducted to maintain participant anonymity and prevent any possible data breaches.

Results

Given the present study's structure with separate blocks intending to identify experiences with anxiety and depression independently of perceptions of the prevalence of such disorders, the findings have been separated into these two categories accordingly.

Perception-Related Findings

While there was no significant difference in the proportions of students who met GAD (23.4%) or MDD (29.4%) criteria during college ($p = 0.115$), the data indicate that Duke students perceive anxiety to be a greater issue among the student population than depression ($p < 0.001$) (Figure A1). At odds with our hypothesis, Duke students' estimates of the prevalence of anxiety in college was higher than the true proportion ($p < 0.001$), while estimates of the prevalence of depression in college were relatively accurate ($p = 0.411$) (Figure A1).

More particular trends in these data are yielded when the perceptions of students who have met the criteria for GAD or MDD in college are statistically isolated. Students who have met the criteria for MDD in college were able to accurately estimate the prevalence of meeting MDD criteria in college ($p = 0.450$), having ever been treated for anxiety ($p = 0.863$), and having ever been treated for depression ($p = 0.973$); however, these students overestimated the prevalence of meeting GAD criteria in college ($p < 0.001$). The same trend

was observed for students who had met the criteria for GAD in college (Figure A2). Additionally, when compared to students who did not meet the criteria for either GAD or MDD in college, students who had met the criteria for either of these disorders perceived GAD prevalence, MDD prevalence, and anxiety or depression treatment as more prevalent among the Duke student body (all $p < 0.05$) (Figure A2). The estimates from students who did not meet these criteria in college was also lower than the true prevalence of meeting MDD criteria in college and having ever been treated for anxiety or depression, although they did still overestimate GAD prevalence (all $p < 0.05$) (Figure A2).

Lastly, although there was no significant difference between the proportion of males and females who had experiences with anxiety or depression (Figure A4), females did perceive GAD and MDD as more prevalent in college than men did (all $p < 0.001$) (Figure A3). The number of non-binary participants did not provide enough statistical power for analysis.

Experience-Related Findings

There were various significant findings regarding the prevalence of MDD and GAD among the student population in relation to demographic factors. When Duke students studying science, technology, engineering, or mathematics (STEM) were compared to students in other fields of study (arts, humanities, and social sciences), non-STEM majors reported having ever been treated for anxiety at a greater rate than STEM majors ($p < 0.001$) (Figure A5). The proportion of Duke students with small social circles who met the criteria for MDD in college was higher than for students who reported having large social circles ($p = 0.004$) (Figure A7). Also, Duke students who reported having no association with religion were more likely to have met the criteria for GAD in college ($p = 0.046$) (Figure A9). Lastly, Duke students whose family income was $85,000 or less were more likely to have met the criteria for MDD in college ($p = 0.002$) (Figure A10). It is worth noting that of all demographic subsets, students in this lowest family income range had one of the highest proportions of students who met these criteria.

In addition to these findings, there were other statistically non-significant results that were noteworthy. For example, students involved in two or fewer school activities were no more likely to have met MDD ($p = 0.484$) or GAD ($p = 0.448$) criteria during their college career than more involved students (Figure A6). Similar proportions of students who self-identified as white or as a different race met MDD ($p = 0.722$) or GAD ($p = 0.338$) criteria in college (Figure A8). Lastly, although the comparison of the proportion of students that had ever been treated for depression to the proportion that had met MDD criteria in college was non-significant ($p = 0.420$), a greater proportion of Duke students had ever been treated for anxiety than had met GAD criteria in college ($p = 0.017$) (Figure A11).

Discussion

Validation of Hypotheses

The original hypotheses included an overall underestimation of the prevalence of MDD and GAD among the Duke student body, a greater prevalence of these disorders at Duke compared to the American population of similarly aged persons, and a greater prevalence of these disorders for students less involved with school activities and social circles. These hypotheses were only partially validated, as some of the data directly contradict our initial predictions. The hypotheses that were supported by the data include a greater likelihood of mental illness for students with smaller social circles and a greater prevalence of mental illness among Duke students when compared to similarly aged individuals in the American population. Data from the National Institute of Mental Health's 2020 Census indicate that among the adult population, persons aged 18-29 were the most likely to have had MDD in the past year (15.2% of individuals) (U.S. Department of Health and Human Services, 2020). While these data cannot be directly compared to the present study given the various amounts of time that students were reporting on (i.e. Freshmen in the study had only been in college for 0.5 years whereas Seniors had been in college for 3.5 years), 29.4% of Duke undergraduates indicated that they had met the criteria for MDD in college. This is nearly double the one-year prevalence of MDD for 18-29 year old Americans, and given the high proportion of Freshmen and Sophomores who participated in the study (78.8% of the sample) it can be inferred with relative confidence that the prevalence of MDD among the Duke undergraduate population is greater than that of American individuals

aged 18-29.

For the same age range, 2.0% of individuals had experienced GAD and 22.3% had experienced any form of anxiety disorder in the past year (U.S. Department of Health and Human Services, 2020). 23.4% of participants in the present study indicated that they had met the criteria for GAD at some point in their college career. These data clearly indicate that GAD is a greater issue among Duke undergraduates than it is among the general American population of 18-29 year old individuals, but the same statement cannot be made for other forms of anxiety given this study's focus on GAD. These results for GAD and MDD are especially concerning when one considers the fact that Duke University provides free counseling to students, a service which may not be available to large portions of the American population. Lastly, it was identified that the rate of comorbidity of GAD and MDD within the sample was consistent with that of the greater American population (U.S. Department of Health and Human Services, 2020).

While these predictions may have been accurate, there were numerous predictions made that were not supported by the data. Primarily, we hypothesized that Duke undergraduates would underestimate the prevalence of mental illness symptoms as reported by the study's participants. Validation of this prediction would have supported the need for a greater presence of "You are not alone" dialogues and similar campaigns that work to advertise the commonality of mental illness. While underestimations were found for three of the conditions in students who had not met MDD or GAD criteria during college, the data were not supportive of this original prediction, as there was no significant difference between the entire sample's predictions of the prevalence of MDD and the true prevalence of this disorder at Duke and students actually tended to overestimate the prevalence of GAD. This indicates that students have a somewhat accurate perception of the proportion of their peers that are suffering from such disorders. Whether or not students recognized that this prevalence was greater than the average for the American population was not assessed by this survey but is a data point that should be collected in future inquiries into this topic. It should also be noted that low involvement in school-related activities was not statistically correlated with more experiences with mental health issues as was predicted based on previous research into mental illness correlates.

Findings and Implications

The data obtained from Duke undergraduates revealed a multitude of complexities regarding the nature of mental illness among the student body as well as potential student populations which may benefit from greater administrative outreach. The most apparent and socially relevant finding is that MDD and GAD are likely larger issues at Duke than they are among the similarly aged American population. The cause of this discrepancy cannot be identified from the data collected, but the myriad of college-related stressors such as financial instability, social media-induced self-deprecation, overwhelming academic rigor, and separation from one's family are likely contributors. This finding justifies the already strong presence of mental health counseling programs on campus such as CAPS and DukeReach.

Additionally, an intriguing finding was that Duke students heavily overestimated the prevalence of GAD amongst their peers. Discussion of stress due to courses and extracurriculars are not uncommon amongst this student body. This consistent, self-perpetuating dialogue may contribute to deceptively large perceptions of the prevalence of anxiety disorders, especially when these discussions turn into competitions over who is the most involved or busy. This may be an aspect of the undergraduate student culture that requires attention and remedy.

Another important finding involves the difference between perceptions of mental illness based on one's previous experience with such disorders. Students who had met the criteria for GAD and/or MDD in college not only accurately predicted students' experiences with depression and anxiety, but these estimated proportions were greater than those for students who had not met these criteria. This shows that students who have suffered from these disorders in the past may have a greater ability to identify symptoms of these disorders in their peers and may have a greater overall perception of the mental health of other students. This awareness indicates that these students could support the combatting of student mental illness. While some students with a history of mental illness may choose to distance themselves from this issue for their own well-being, others may want

to apply their newfound sense of empathy for students who are struggling. If these individuals were to help lead mental illness awareness campaigns or programs such as Duke's Peer Support Program, their accurate perception of the state of mental health amongst their own student body may contribute to the success of these endeavors. Alternatively, these students' high (yet accurate) estimates of mental illness prevalence could potentially be attributable to the false consensus effect, or a generalization of their own experiences to the entire Duke student body.

The present study also provided helpful information regarding student groups who may be most at-risk for experiencing mental health issues. The findings that non-STEM students were more likely to have been treated for anxiety, students with small circles were more likely to have met MDD criteria in college, non-religious students were more likely to have met GAD criteria in college, and students from the lowest U.S. tax bracket were more likely to have met MDD criteria in college provide potential opportunities for Duke to better tailor its student mental health outreach programs. By bolstering advertising of counseling services, the Peer Support Program, and other academic and health-related resources to students who fall into these categories, efficient mental illness counseling can potentially be provided to a greater number of students in need. It should also be noted that the counseling provided should not necessarily be confined to college experiences, as providing students with useful skills such as mindfulness has been shown to provide long-term mental health benefits that could endure past the end of their college career (Baer, Lykins, & Peters, 2012). Additionally, the non-significant statistical relationships, such as the relationship between mental illness prevalence and racial identity or level of school involvement, should also be used to provide context to the changes that are made to student outreach. For example, while low school involvement may be a correlate of mental illness in the general population, this does not seem to be the case at Duke. Therefore, focusing efforts on reaching out to students who are less involved in university-sponsored activities at Duke may not be an effective approach in alleviating student suffering.

Finally, a question at the end of the survey asked students for any resources that Duke could have implemented to alleviate their mental stress, and the most common responses involved the availability of CAPS (hours open and appointment count), mental health support groups, and wellness days integrated into the school schedule. Students often complained about having to wait weeks for initial appointments at CAPS when the state of their mental health required immediate attention. To alleviate these worries, it may be beneficial for CAPS to recruit a larger number of psychologists and to encourage and advertise the need to reach out early if a student is beginning to experience symptoms of mental illness. Additionally, the creation of mental health support groups may be beneficial for the student body, but there may also be issues of anonymity that prevent students from participating. Further interest in such programs should be gauged before support groups like these are implemented. Finally, the implementation of wellness days into the academic calendar was a relatively popular recommendation. Daily schedules involving abnormally early or late work have been shown to have deteriorating effects on sleep quality and mental health (Lin et al., 2011). The implementation of occasional days off to reset one's agenda of coursework and extracurriculars may help combat atypical student schedules and contribute to positive effects on mental health.

Limitations and Future Directions

While the present study helps to shed light on issues surrounding mental health at Duke, there are notable limitations in how the data were collected and how they can be used. Primarily, the data were obtained while students were still under state- and university-mandated restrictions about one and a half years after the COVID-19 pandemic's inception. One could infer based on Hall-Lande et al.'s (2007) findings that the inability to sufficiently interact with one's peers due to sanctions put in place to safeguard the public's health would have a negative effect on student mental health. While we estimated that MDD and GAD are greater issues at Duke than they are among the general American population, there is potential for this finding to be refuted based on future data regarding Americans' mental health during the height of the COVID-19 pandemic. Additionally, there were certain demographic groups that, while present in the Duke undergraduate population, were missing

from the participant pool in this study. This not only limits the number of analyses that can be conducted but also potentially threatens the generalizability of the data to the entire Duke undergraduate student body. However, these demographic groups, such as Native American students, often made up fewer than 1% of the student body, therefore bolstering reliability in the data obtained. While perspectives of these students are valuable and would have contributed to a greater understanding of student mental health, their absence does not create sufficient doubt in the study's generalizability. Similarly, the possibility of selection bias in the data set must be considered, as students who have suffered from mental illness in college may have been more likely to participate in the study and share their experiences than students who had less familiarity with these illnesses.

The findings documented here should also be used with caution in any future considerations of amendments to student mental health counseling programs. Since this study was conducted during the COVID-19 pandemic, some of the results may not be applicable to students in a few years who are no longer experiencing the negative effects of health and safety restrictions. In fact, an optional question at the end of our survey asked students for any potential contributing factors to their experiences with mental illness symptoms, and 31 students responded with an answer related to COVID-19. The culture of the student body at Duke is also very subject to change as institutional adjustments are made at Duke's administrative level. For example, the removal of Greek Life from Duke's campus and the installation of the new QuadEx housing system have the potential to shift student sentiments and emotional well-being. For these reasons, it is recommended that surveys similar to the one used in this study are repeated at Duke every four years (when a new undergraduate student body is present).

Lastly, one of the most apparent limitations of this study is its use of self-reporting to obtain data. The study asked participants a series of questions that allowed us to estimate each student's likelihood of having had MDD or GAD during college. These disorders can only be properly diagnosed by licensed psychologists and doctors, hence the present use of the verbiage "met MDD/GAD criteria in college" as opposed to "have had MDD/GAD in college." This raises the question of the reliability of such self-reports of past experiences. However, the reliability and validity of self-reports of mental illness symptoms has been previously established in psychology literature (Meyer et al., 2020). Therefore, while the data should not be viewed as an exact proportion of students experiencing these disorders, it is likely that they are a proper reflection of the general state of mental health among the Duke undergraduate student body. A similar concern is students' ability to comprehend the purpose behind the language used in the DSM-5. For example, the criteria for GAD include excessive worry and an impairment of functioning, but students answering this questionnaire may not be able to understand the clinical significance and threshold for these symptoms. This misunderstanding could potentially skew prevalence data in either direction, but it is more likely that it would have inflated the proportion of students who have reported suffering from these conditions.

Concluding Remarks

The purpose of the present study was not solely to analyze the state of student mental health at Duke University, but to also call greater attention to the rising issue of college student mental illness and to propose a mechanism for change. It is our hope that the administrative bodies of Duke's mental health counseling programs will take these findings into account in their future approaches to student outreach and treatment given the readily apparent issues of depression and anxiety among the student body. Additionally, with further funding and institutional permission, our goal is to expand the present survey to include other universities in the United States. As stated earlier, it is the researchers' opinion that it is best to analyze the mental health of each university's student body individually due to cultural factors that may skew data when multiple universities' datasets are combined. The comparison of data between universities will also help identify whether this is a necessary distinction to make. Finally, whatever changes may be made to the mental health counseling programs of the universities where these studies are conducted should be further analyzed to determine their efficacy and identify what institutional changes are most effective in alleviating student suffering.

References

1. An, Z., & McDermott, V. M. (2014). The effects of sociocultural factors and perceptions of mental illness on indirect disclosure preferences. *Communication Research Reports, 31*(3), 281–291. https://doi.org/10.1080/08824096.2014.924338
2. American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.). https://doi.org/10.1176/appi.books.9780890425596
3. Baer, R.A., Lykins, E.L.B., & Peters, J.R. (2012). Mindfulness and self-compassion as predictors of psychological wellbeing in long-term meditators and matched nonmeditators, *The Journal of Positive Psychology, 7*(3), 230-238. doi:10.1080/17439760.2012.674548
4. Blanco, C., Okuda, M., Wright, C., Hasin, D., Grant, B., Liu, S., & Olfson, M. (2008). Mental Health of College Students and Their Non–College-Attending Peers: Results From the National Epidemiologic Study on Alcohol and Related Conditions. *Arch Gen Psychiatry, 65*(12), 1429–1437. doi:10.1001/archpsyc.65.12.1429.
5. Casey, B.J., Jones, R.M., Levita, L., Libby, V., Pattwell, S.S., Ruberry, E.J., Soliman, F., Somerville, L.H.. The storm and stress of adolescence: insights from human imaging and mouse genetics. *Dev Psychobiol, 52*, 225–235. doi:10.1002/dev.20447.
6. Ford-Paz, R. E., Santiago, C. D., Coyne, C. A., Rivera, C., Guo, S., Rusch, D., St. Jean, N., Hilado, A., & Cicchetti, C. (2020). You're not alone: A public health response to immigrant/refugee distress in the current sociopolitical context. *Psychological Services, 17*(S1), 128–138. https://doi.org/10.1037/ser0000381
7. Hall-Lande, J. A., Eisenberg, M. E., Christenson, S. L., & Neumark-Sztainer, D. (2007). Social isolation, psychological health, and protective factors in adolescence. *Adolescence, 42*(166), 265-286.
8. Karlin, B. E., Duffy, M., & Gleaves, D. H. (2008). Patterns and predictors of mental health service use and mental illness among older and younger adults in the United States. *Psychological Services, 5*(3), 275–294. https://doi.org/10.1037/1541-1559.5.3.275
9. Kelly, C. M., Jorm, A. F., & Wright, A. (2007). Improving mental health literacy as a strategy to facilitate early intervention for mental disorders. *Medical Journal of Australia, 187*(S7). https://doi.org/10.5694/j.1326-5377.2007.tb01332.x
10. Lin, P. C., Chen, CH., Pan, SM. et al. (2012). Atypical work schedules are associated with poor sleep quality and mental health in Taiwan female nurses. *Int Arch Occup Environ Health, 85*, 877–884. https://doi.org/10.1007/s00420-011-0730-8
11. Meyer, T. D., Crist, N., La Rosa, N. et al. (2020). Are existing self-ratings of acute manic symptoms in adults reliable and valid?—A systematic review. *Bipolar Disorders, 22*(6), 558-568. https://doi.org/10.1111/bdi.12906
12. Schomerus, G., Schwahn, C., Holzinger, A., Corrigan, P. W., Grabe, H. J., Carta, M. G., & Angermeyer, M. C. (2012). Evolution of public attitudes about mental illness: A systematic review and meta-analysis. *Acta Psychiatrica Scandinavica, 125*(6), 440–452. https://doi.org/10.1111/j.1600-0447.2012.01826.x
13. Thakur, V., Jain, A. (2020). COVID 2019-suicides: A global psychological pandemic. *Brain Behav Immun., 88*, 952–953.
14. Thomée, S., Eklöf, M., Gustafsson, E., Nilsson, R., & Hagberg, M. (2007). Prevalence of perceived stress, symptoms of depression and sleep disturbances in relation to Information and Communication Technology (ICT) use among young adults – an explorative prospective study. *Computers in Human Behavior, 23*(3), 1300–1321. https://doi.org/10.1016/j.chb.2004.12.007
15. U.S. Department of Health and Human Services. (2020). Generalized anxiety disorder. National Institute of Mental Health. https://www.nimh.nih.gov/health/statistics/generalized-anxiety-disorder
16. U.S. Department of Health and Human Services. (2020). Major depression. N*ational Institute of Mental Health*. https://www.nimh.nih.gov/health/statistics/major-depression.
17. Wang, M.T., & Degol, J. (2016). School Climate: a Review of the Construct, Measurement, and Impact on Student Outcomes. *Educ Psychol Rev, 28*, 315–352.
18. Whitaker, R. (2005). Anatomy of an Epidemic:

Psychiatric Drugs and the Astonishing Rise of Mental Illness in America. *Ethical Human Psychology and Psychiatry, 7*(1), 23–35.

19. Yorgason, J., Linville, D., & Zitzman, B. (2008). Mental Health Among College Students: Do Those Who Need Services Know About and Use Them?. *Journal of American College Health, 57*(2), 173-182. doi:10.3200/JACH.57.2.173-182.

Appendix

Graphical Representations of Student Response Findings

All error bars are representations of standard error and are not one standard deviation from the mean.

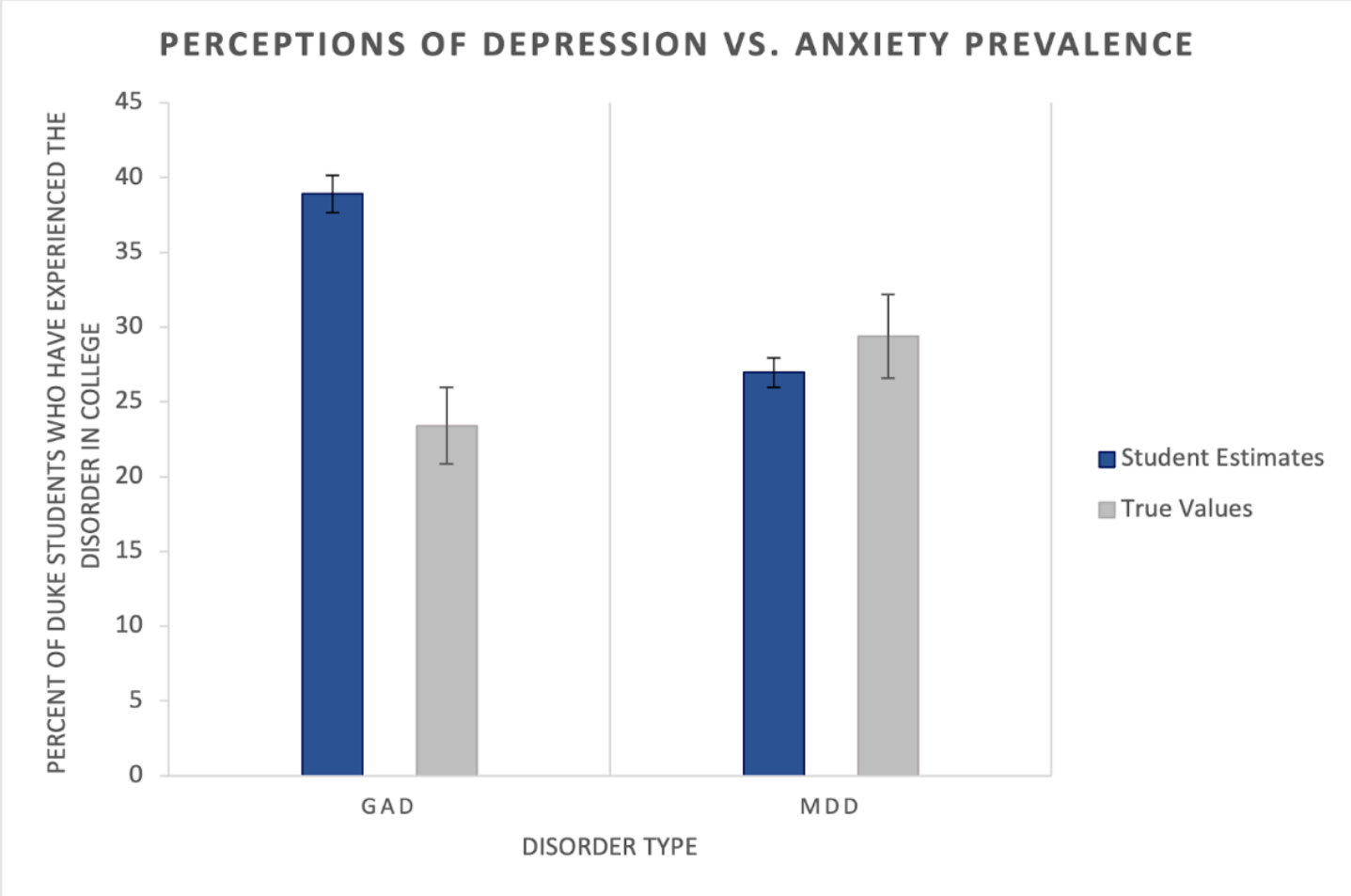

Figure A1. A comparison of student estimates of disorder prevalence among Duke undergraduates to the true prevalence of such disorders among the sample.

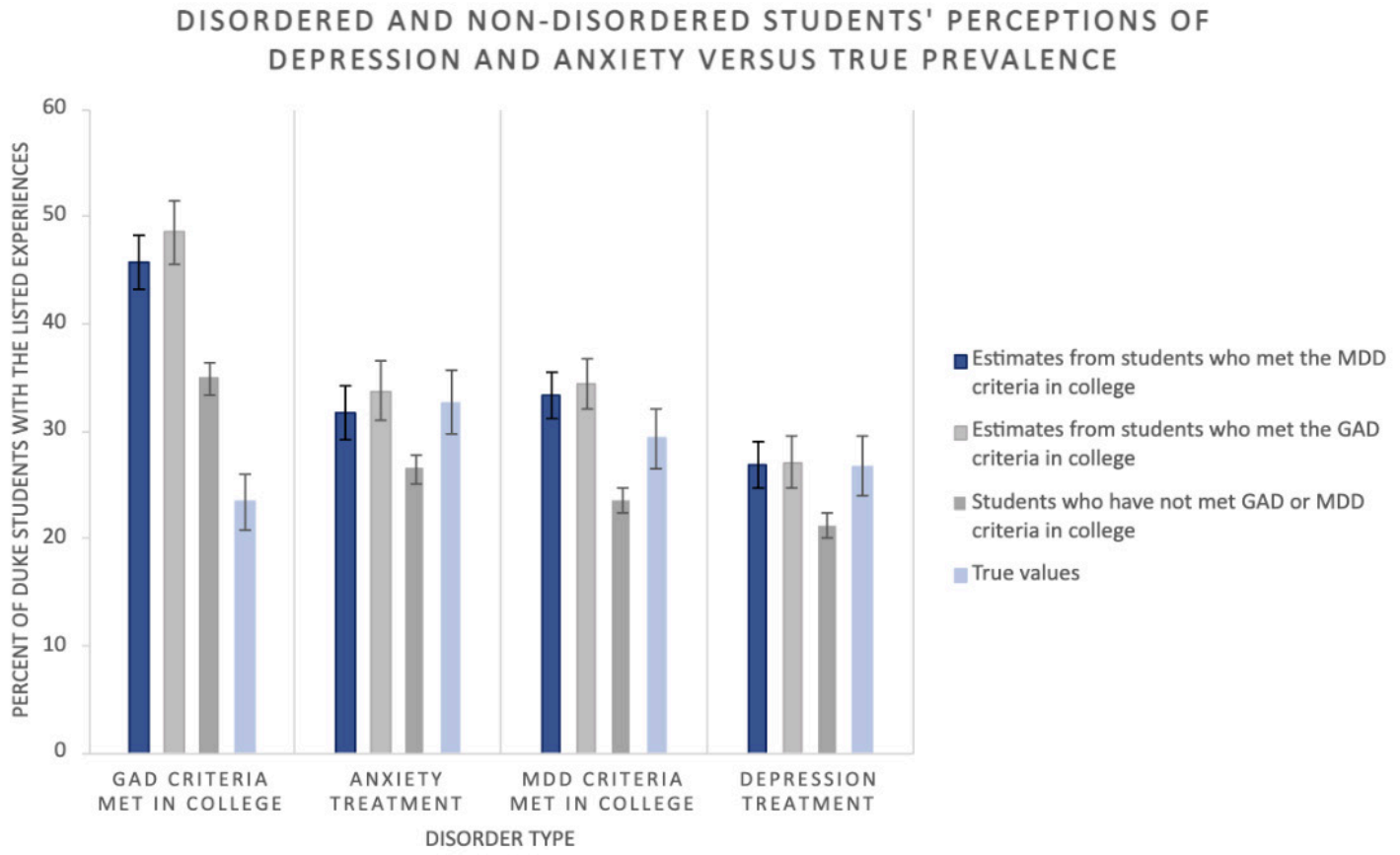

Figure A2. A comparison of prevalence estimates from students who have met MDD criteria, students who have met GAD criteria, and students who have not met either criterion to the true prevalence of such disorders among the sample.

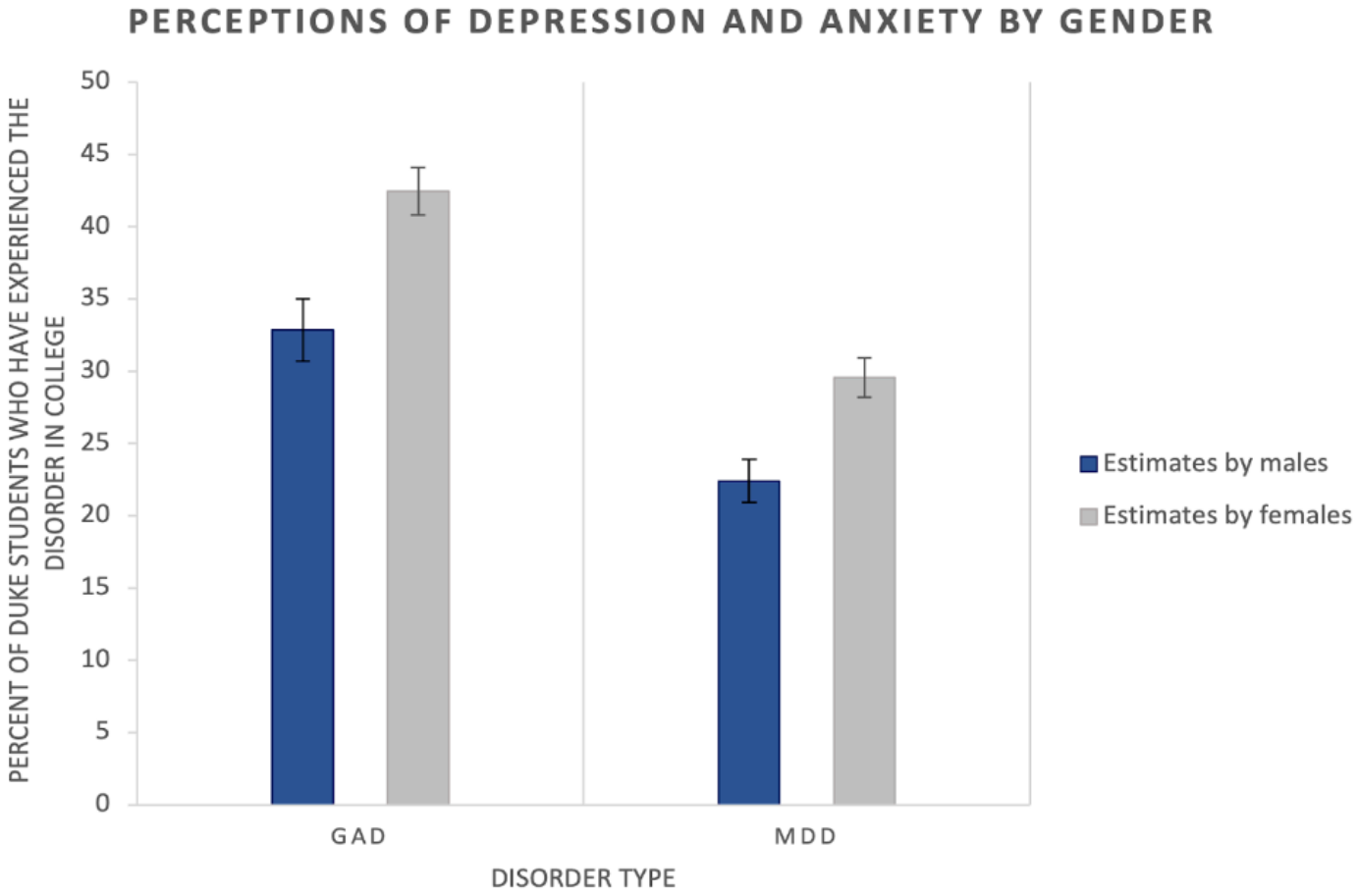

Figure A3. A comparison of prevalence estimates from males and females regarding GAD and MDD in college.

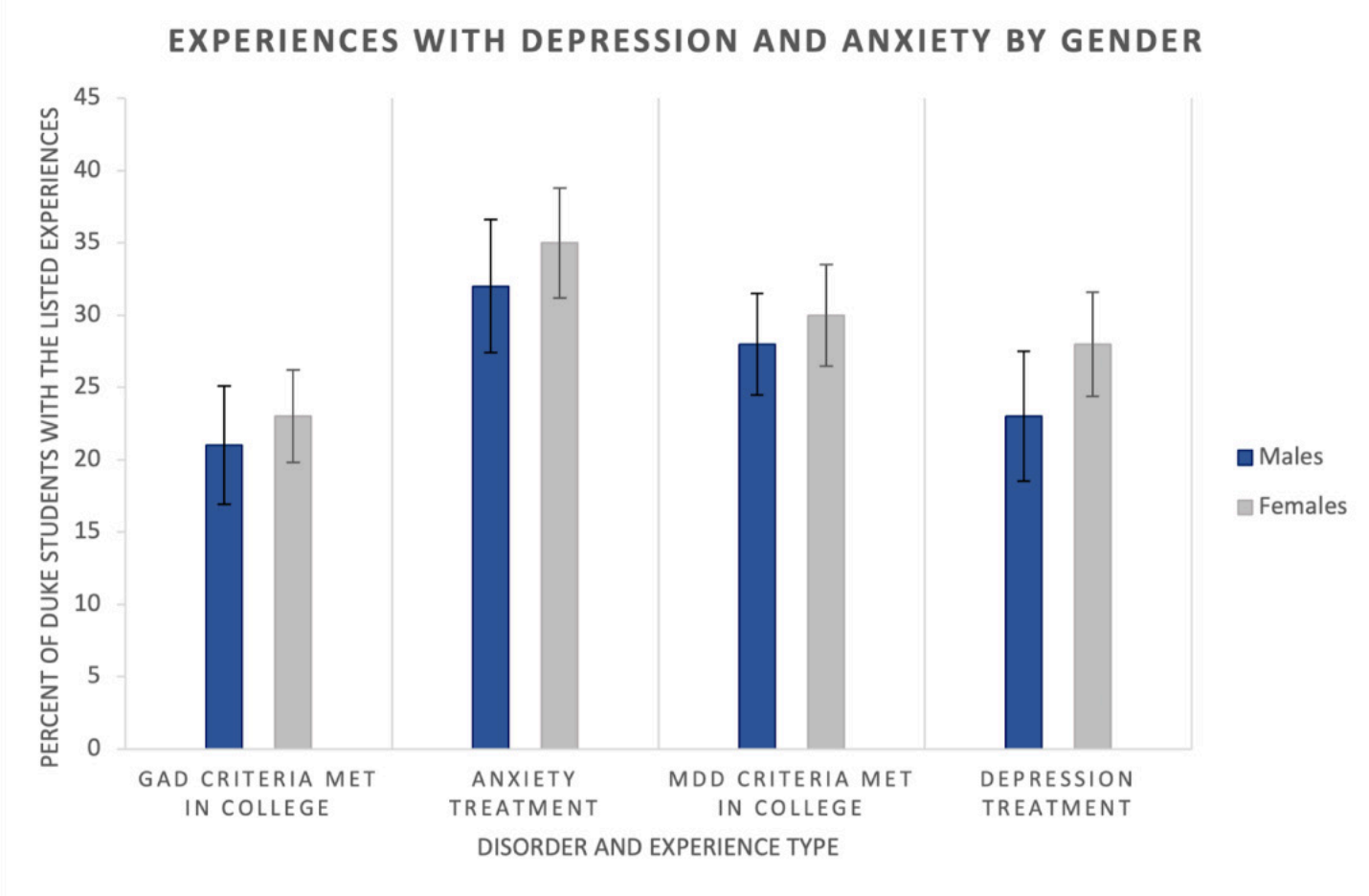

Figure A4. A comparison of the prevalence of anxiety- and depression-related experiences based on Duke students' gender identity.

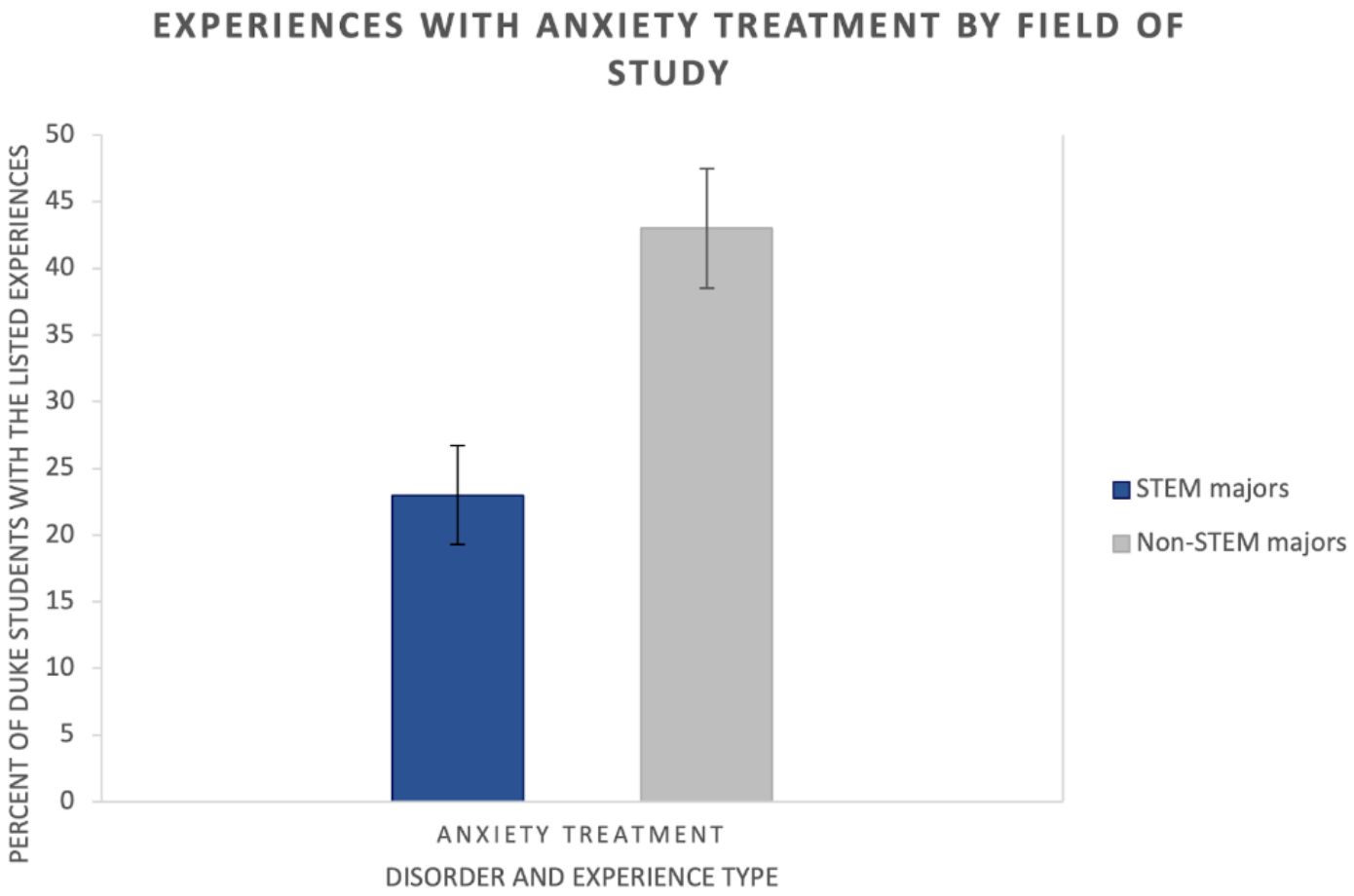

Figure A5. A comparison of the prevalence of clinical treatment for anxiety among Duke students based on their academic field of study.

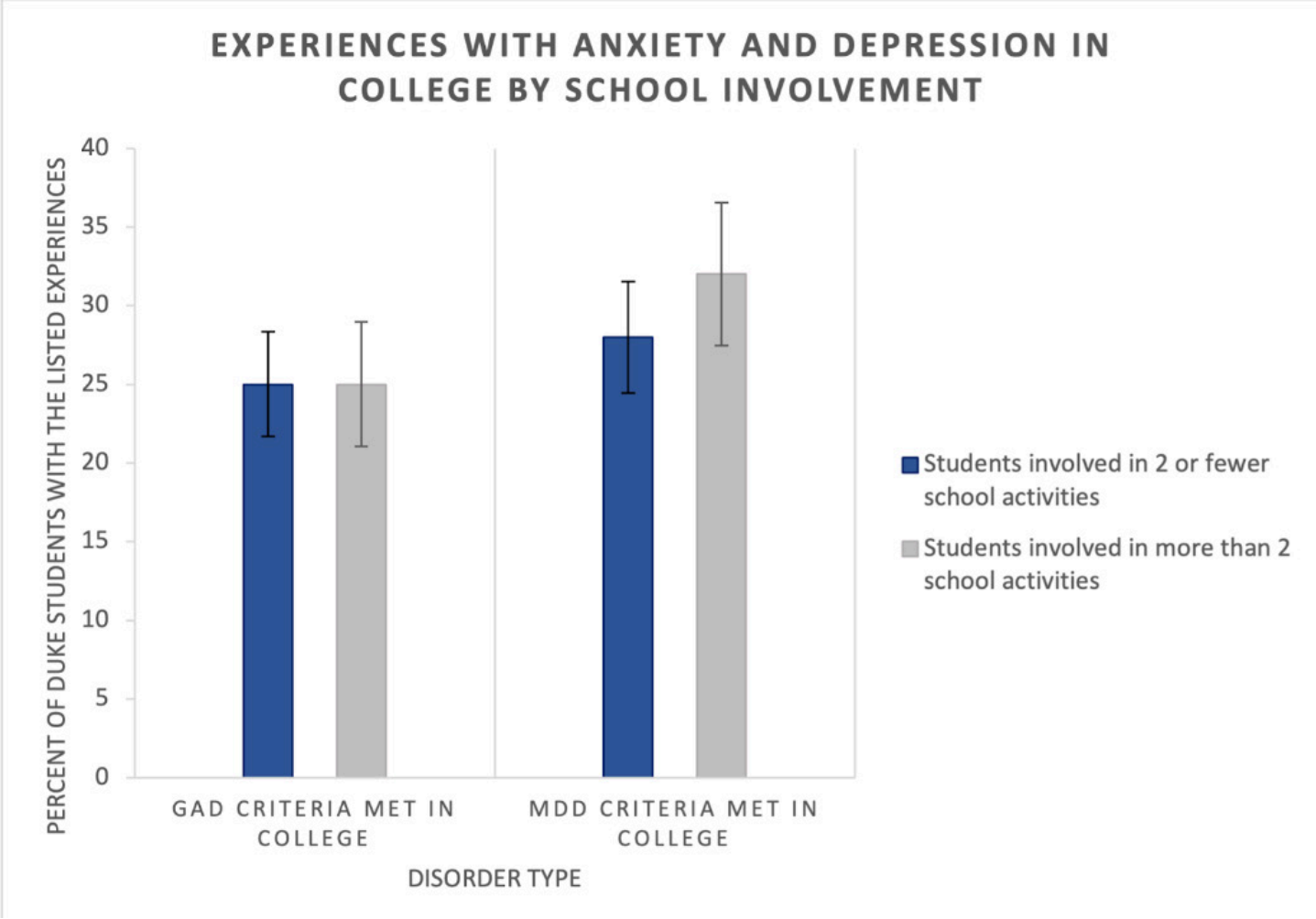

Figure A6. A comparison of the proportion of students who have met MDD or GAD criteria in college based on their level of involvement in school activities.

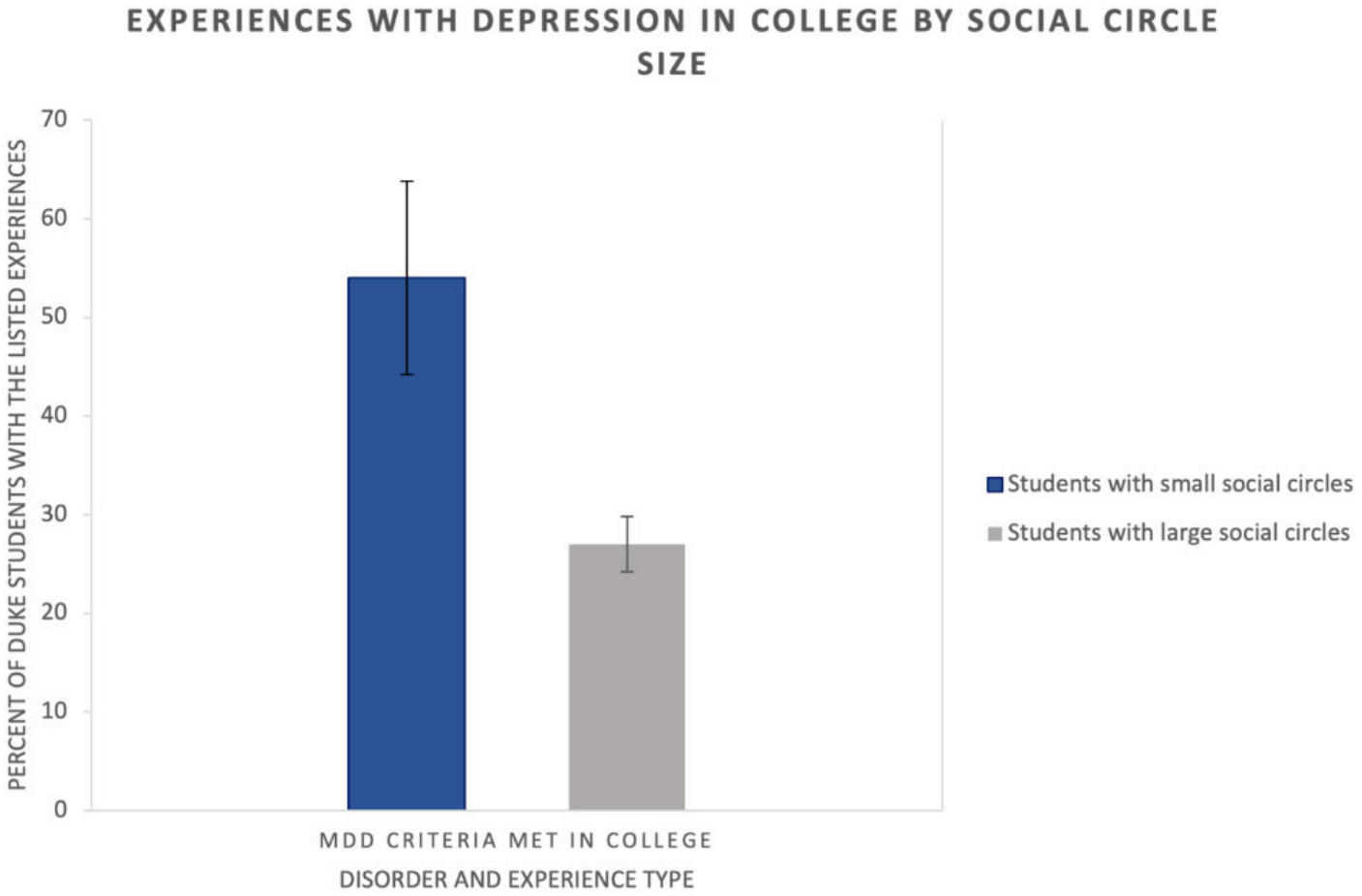

Figure A7. A comparison of the proportion of students who have met MDD criteria in college based on the size of their social circles.

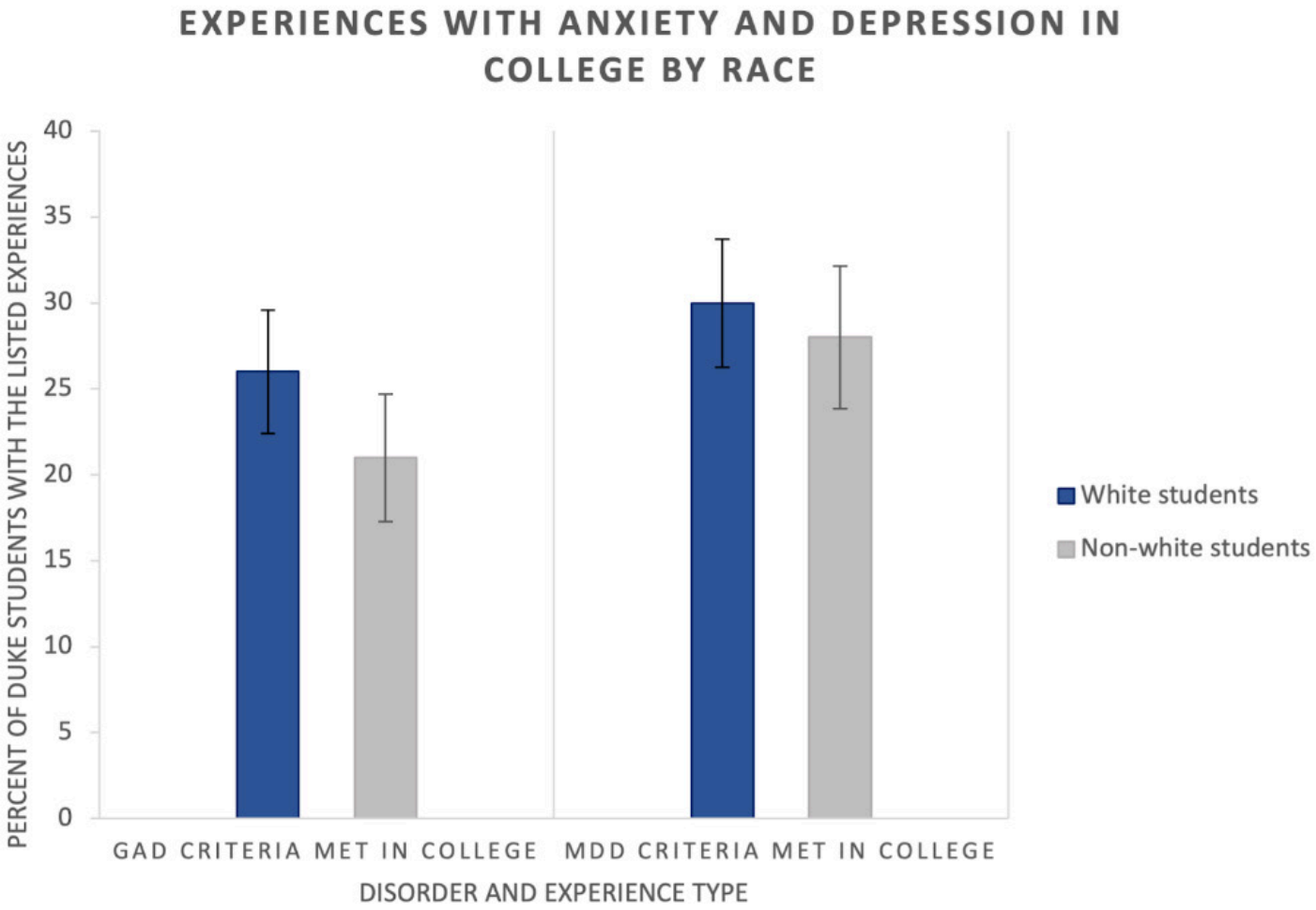

Figure A8. A comparison of the proportion of students who have met GAD or MDD criteria in college based on their self-identified race.

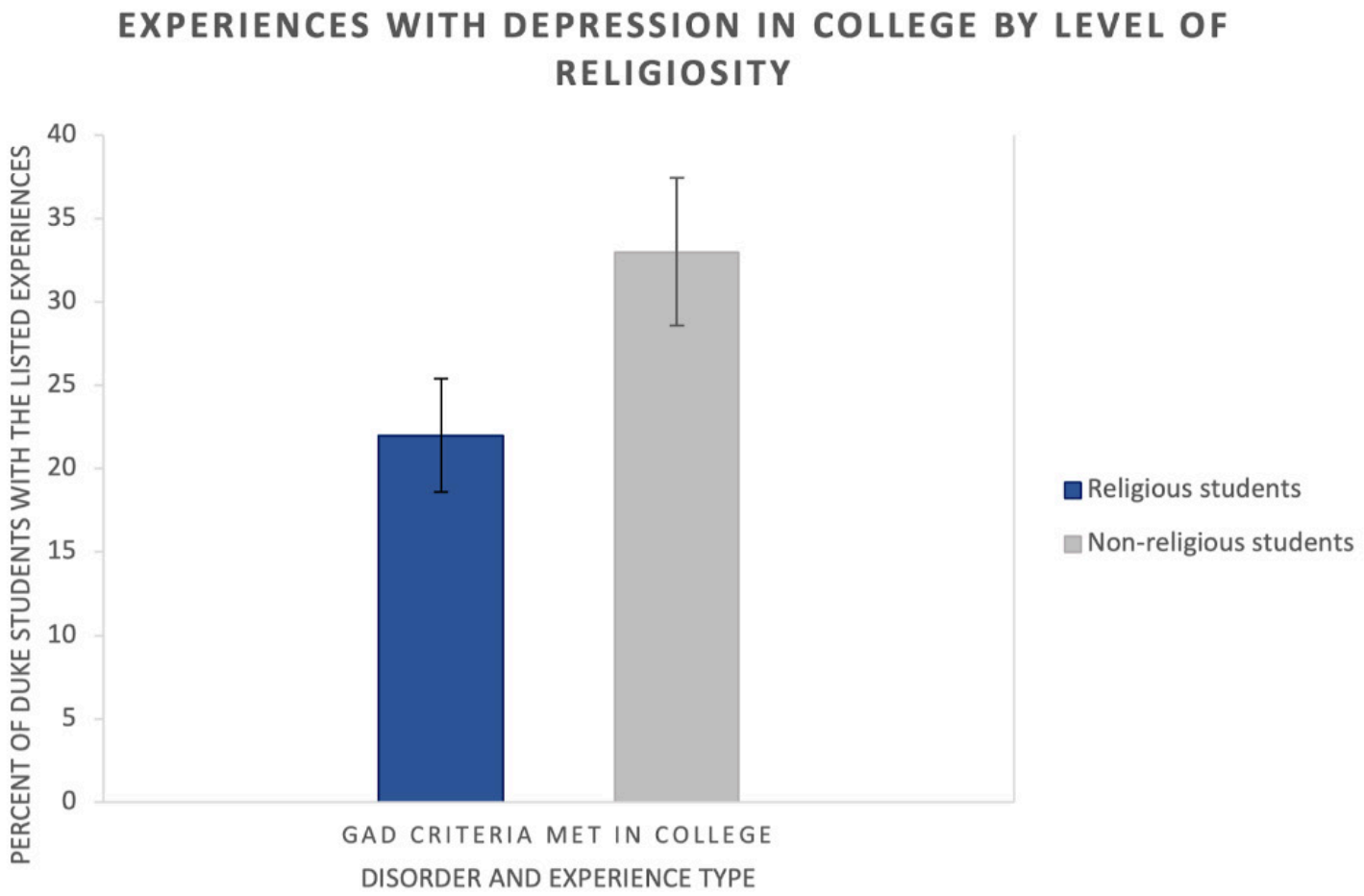

Figure A9. A comparison of the proportion of students who have met GAD criteria in college based on whether they consider themselves religious.

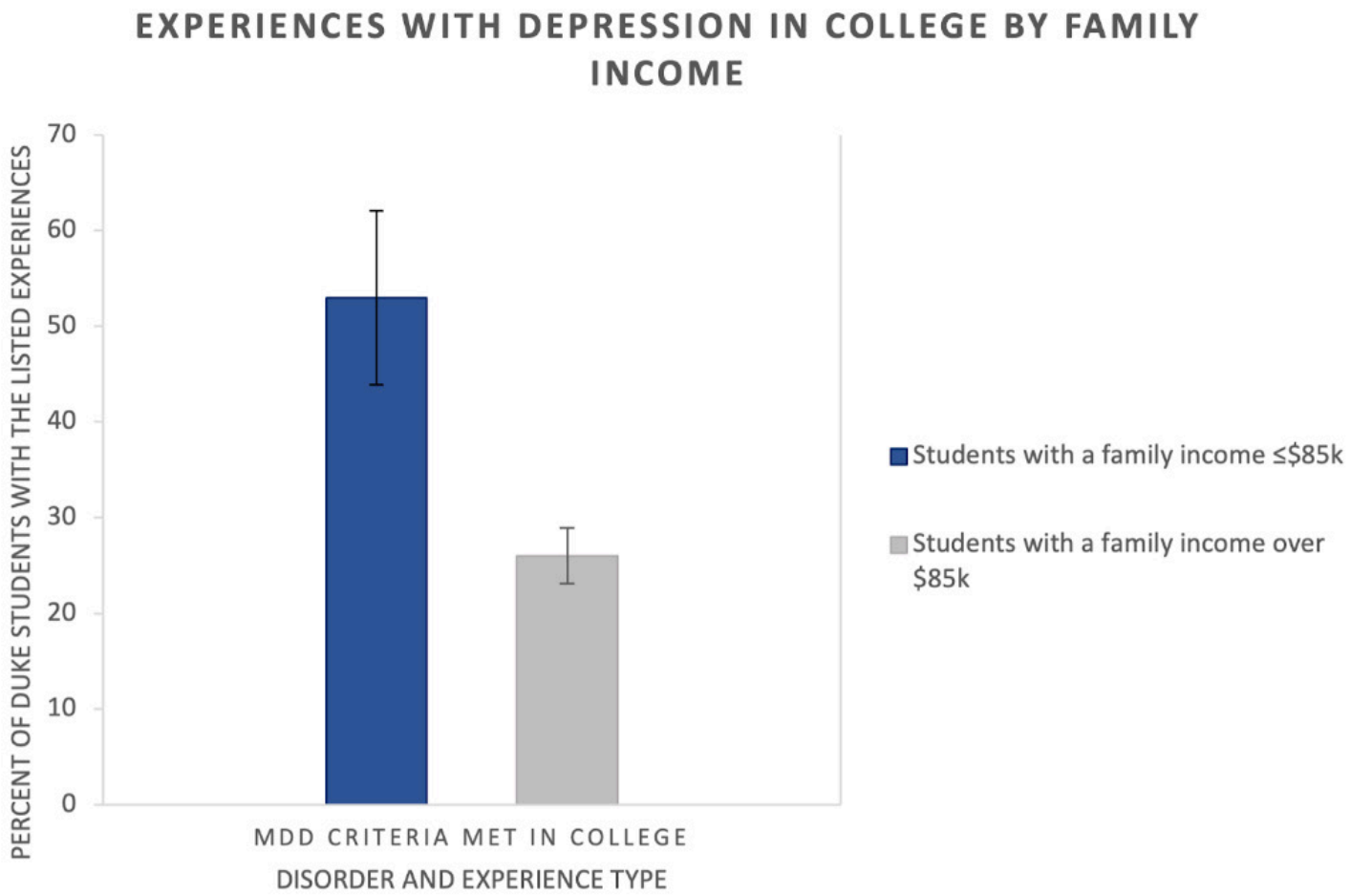

Figure A10. A comparison of the proportion of students who have met MDD criteria in college based on their family's estimated family income.

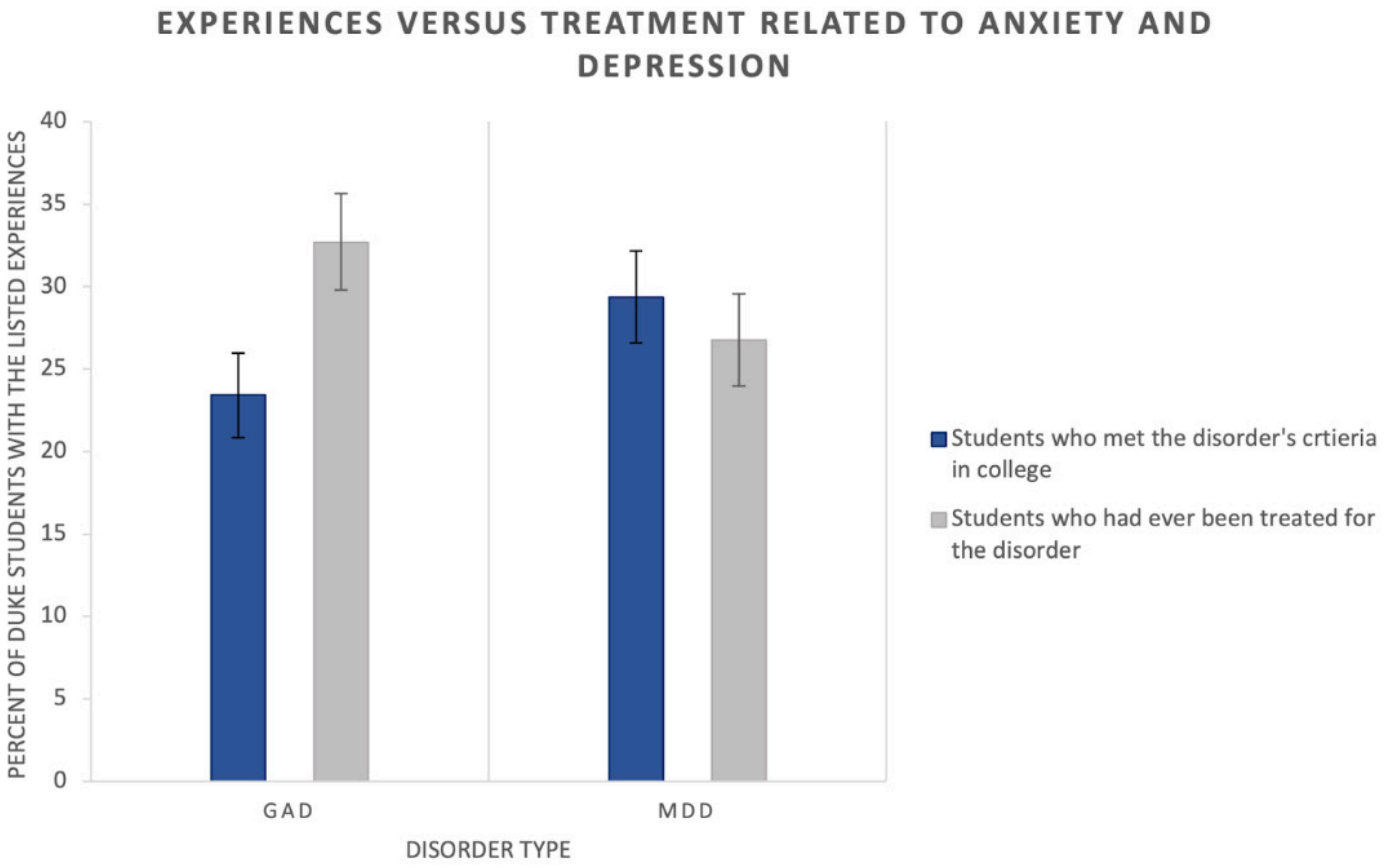

Figure A11. A comparison of the proportion of students who have met MDD or GAD's criteria in college to students who have ever been treated for these disorders.

Data Corroboration of The Catastrophic Chernobyl Tragedy Using Arc-Length Estimate Conjecture

Manan Roy Choudhury, Anurag Dutta, Arnab Kumar De

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Article Synopsis

Estimated losses in business organizations soar up to 4–5% of their revenues due to fraudulent transactions. FICO found that 4 out of 5 banks in their survey have experienced Data Fraud in the last year. 11% of the These several fraud cases happening in our day to day lives need to be tackled and stopped immediately. But there are many problems with the current data validation laws. Some of these problems are extremely high false positives, missing insights from unstructured data, delayed alert mechanism, missing omni channel coverage, lack of insights from analytics, etc.

But Arc-Length Estimate formula solves most of these problems faced by the old data validation laws. Arc-Length Estimate Conjecture is a data validation formula that can accurately detect anomaly and fairness of datasets. There are many perks of using Arc-Length Estimate like it provides us double verification, it's easy to implement, minimum possible error, provides error bounds, provides confirmation range and works well with almost all datasets. The dataset of the concentration of I131 (Iodine 131) and Cs134 (Cesium 134) after the Chernobyl nuclear cataclysm is validated using Arc-Length Estimate Conjecture based on the concentration of aerosol particles which were measured in some specific location in Europe after the incident. The results obtained were overwhelming and accurately matched the practical observations collected from the event sites.

Data Corroboration of The Catastrophic Chernobyl Tragedy Using Arc-Length Estimate Conjecture

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Abstract

Nuclear power is perhaps one of the biggest keys to empowerment for any country. In fact, nuclear power has been the backbone of the international superpowers like the US and USSR since the 20th Century. Alongside its merits, nuclear power carried some demerits propping on it. One of the biggest amongst all was the Chernobyl nuclear cataclysm that occurred on the 26'th April 1986. Within hours of the explosion, nuclear radiation blanketed the whole city of Pripyat, Ukraine. In this paper, we have developed a notion namely "Arc-Length Estimate Conjecture" to detect anomaly, and fairness in data sets. If an anomaly is found, the Arc Length Estimate Conjecture is capable of dissecting the anomality in magnitudical terms. Further we have analyzed the Radiation that emerged from the Chernobyl Catastrophe by looking at the concentration of radioactive elements that were measured from different parts of the world. We have considered the elements I131 and Cs134 as elements of utmost importance and have tried to verify the data put forward by USSR regarding the radiation emission level of these radio actives. social-circles, and non-religious students. The results of this study and related studies to follow will be used to inform improvements to mental health counseling and outreach to at-risk individuals at universities across the United States.

Keywords: Chernobyl, Benford's law, Zipf's law, Nuclear Reactions, Chi-Square Test, Mean Absolute Deviation

1. Introduction

Nuclear Energy, it's the term given to the energy present in the core of any atom. Briefly, it's the energy generated in the process in which two or more particles collide amongst each other resulting in generation of a new particle along with enormous energy being dissipated. These processes can be of various types, the most significant ones amongst them are Nuclear Fusion and Nuclear Fission[1]. The incident witnessed by Chernobyl Power Plant on the night of 26'th April '86 was withstood on Nuclear Fission. It was (1)RBMK reactors that used

(1) RBMK (reaktor bolshoy moshchnosti kanalnyy) is a Generation II type reactor that was one of the most widely used nuclear reactor. One of the astonishing facts that prevailed between nuclear scientists of that time was – "An RBMK reactor can never explode". Inspite of its worldwide acceptance, it had something disastrous brewing inside it. RBMK reactors were very unstable at low power, which was one of the reasons behind the mishap.

to operate in many Nuclear Power Plants[2] throughout the USSR at that time. On the, night of 26'th April '86 a misconduct happened during the conduct of some safety tests, which resulted in the collapse and explosion of the 4'th reactor of Chernobyl Power Plant[3]. The cloud behind this treacherous incident started condensing a lot before. The Chernobyl Power Plant (officially known as Vladmir Lenin Nuclear Power Plant) was commissioned in the mid 70's. For any nuclear power plant to be made completely operational, first it has to pass a lot of safety tests. The 4th reactor of the Chernobyl Power Plant[4] went into operation on December 20, 1983.

Now, in actuality the Construction wasn't complete including the safety tests. Numerous other safety tests occurred after the December of '83. One of them was the Turbine run – down energy capability[5] test which was scheduled to be performed on 25'th April 1986 in the Day Shift. To perform the test, the power generated by the plant (3200 MW) was needed to be cut down to (200 MW). During this process of lowering the power, while the power generation[6] was set to 1600 MW, a grid controller in (2)Kiev called for the plant Director requesting no further reduction in the Output Power until midnight. Thus, the test was scheduled on the night shift under the supervision of Comrade Dyatlov. Now, in the Chernobyl Nuclear Power Plant, the fuel used was 92235U with Graphite as Moderator and (3)RBMK type control rods. One of the most important by – product produced by this type of reactor were Xenon. [7]E. I. Grishanin, a fellow at the Russian Research Center Kurchatov Institute in his paper "The Role of Chemical Reactions in the Chernobyl Accident" mentioned – "Prior to the tests, the reactor was strongly poisoned with xenon, which, according to our estimates, temporarily decreased the reactivity margin by 2.9% and significantly increased the steam effect of reactivity". Conducting the Turbine run – down energy capability test in such a situation was known beforehand to cause a catastrophe. The test started at 1:23:04 hours on the night and a series of wrong decisions was taken which resulted in a sudden surge in power from 204 MW to 33,000 MW and at 1:23:40 hours, Senior Supervisor, Aleksandr Fyodorovich Akimov pressed the (4)AZ – 5 button of the Chernobyl Power Plant but due to the presence of the Graphite in the tips of the control rods, instead of stopping the reaction taking place at the core, it surged. Finally, at 1:23:45 hours, Chernobyl witnessed that incident which was going to change it's demographics for years. Within hours, radiation from the [1]molten lava-like fuel spread throughout the nation. Later the people were evacuated and [8]many lost their own lives, even many lost their family members. In 1987, the government constructed a dome on the remains of the reactor which is till date kept under supervision. The waste clean – up process is scheduled to be complete by 2065[9].
In the Chernobyl Nuclear Power Plant incident[10][11], there were many stages from where the destruction that happened could be reversed[12]. Given below is a tabular form of some of them.

(2) Capital of Ukraine.
(3) In such type of Control Rods, the tip was made of Graphite while the rest part was made up of Boron.
(4) It is a button in the RBMK type reactors to immediately stop nuclear fission.

Table 1. Crucial Moments that led to the CNPP incident

Year	*Month*	*Date*	*Time*	*What was done*	*What should have been done*
1983	December	31	09:48	Fake Certification of Completion of construction of CNPP	No Fake Certification should have been given
1986	April	25	10:00 to 22:00	CNPP was made to operate in a reduced output of 1600 MW, resulting in Xenon accumulation in the core.	CNPP should have been made to operate at the normal output of 3200 MW.
1986	April	26	1:20	Output power was lowered after making the reactor run at the reduced output for 12 hours.	The test would not have been performed.
1986	April	26	1:23	Power was increased from NULL.	The reactor should have been shut down
1986	April	26	1:23:45	Scientists and officials argued on the cause of the explosion.	Evacuation should have been started.

Later, analyzing the Radiation by looking at the concentration of I131 (Iodine – 131)[13][14] measured from different parts of the world, we verified the justification of the concentration dataset.
Now, we will analyze the name of the formula proposed in this paper. Zipfian distribution[15] is a very well-known statistical distribution. In this paper, the graph of Zipfian distribution is taken as a reference and the arc length of the distribution is calculate from x=1 to x=9. This, basically justifies the name behind the formula proposed in this paper i.e., "Arc-Length Estimate Conjecture". In this paper, the Arc-Length of the curve is calculated in two different ways and they are equated. To be precise, the final equation will contain some errors and thus, we have also calculated the absolute percentage error that will be generated in the mentioned process[16].

From the next section, supporting pillars are erected to standardize our conjecture, especially by making use of the prevalent Zipf's Law, which is a statistical law known with prominence to resolve issues specific to linguistics. Section 3 marks the notion, that have been designed to validate data chunks, and Section 4 leverages the notion, of its potential to distinguish fallacies. For the same, we have used the data, published openly by the Soviet Union, back then in 20'th Century. The main focus is to validate the dataset, and jot down the fallacies if found following the data. Section 5 proves the notion – "Arc Length Conjecture" to be juxtaposed, and potent with the capability to emboss fraudulences if found. For the same, a linearity check is run through the result, cross validating the latter using the prevalent Zipf's Law. Section 6 concludes the work, and it's merits in comparison to the prevalent statistical laws.

2. Zipf's Law

An empirical law states that the frequency of occurrence of certain events and their rank exists in inverse relation. Zipf's law was originally formulated in order to meet various purposes in the domains of linguistics and literature. The original law states that in a given unbiased corpus of natural language heap, the frequency of occurrence of any word is inversely proportional to its rank.
This phenomenon was sought to be first noted in the 1930s by Kingsley Zipf who proposed it. The French stenographer Jean Baptiste Estoup (1868-1950) was the first person to notice this regularity which is maintained by almost all unbiased corpus of natural language da[illegible]erman physicist Felix Auerbach also noted this phenomenon in 1913.
Mathematically,

$$f \propto \frac{1}{r}$$

where f is the frequency and r is the rank.
[9]It can also be stated as – "The Product of frequency and rank of any linguistic or sociological data sets is constant"
Zipf's law has varied applications in different domains. Some of them are:

- Intrinsic uses in fields like Information Theory.
- A very well-known law in domains like finances and forensics.
- Varied usage in linguistics, language, and literature.
- Used entropy coding methods in data compression and mining.
- Forage of extra-terrestrial intelligence.

3. An Unified Notion

In this Section, we have introduced a new methodology namely - "Arc-Length Estimate Conjecture" for validating a chunk of data.
According to Zipf's Law,

$$f(r)\times r = c^2$$

where,
fr is the frequency corresponding to rank r.
c^2 being a positive constant.

If we consider, the rank to be acquainted along the x – axis, and the frequency along the y – axis, their 2 – D plot on the x – y coordinate system will be a Rectangular Hyperbola with the x, and y axes as their asymptotes.
So, $f(r)\times r = c^2$.
The set of Discrete point(s) within domain corresponding to fx will be
$\{(1, f(1)), (2, f(2)), (3, f(3)), (4, f(4)), (5, f(5)), (6, f(6)), \ldots, (9, f(9))\}$.
Graphically,

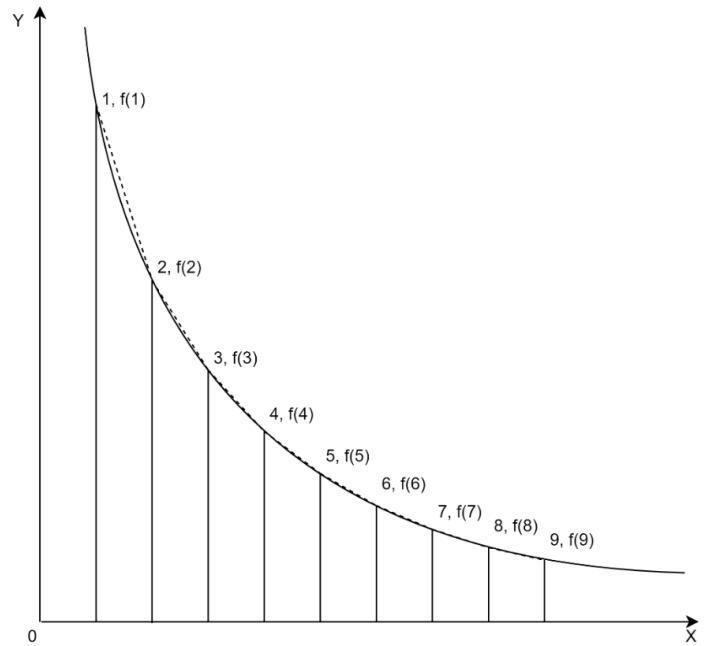

Fig. 1. Graphical Representation of fx×x=c2 along with the discrete points.

Considering the discrete points,
Length of straight line joining $(i, f(i))$ and $(i+1, f(i+1)) = \sqrt{1 + (f(i+1) - f(i))^2}$
So, total length, L will be $\sum_{i=1}^{8}\left(\sqrt{1 + (f(i+1) - f(i))^2}\right)$

Also, by Zipf's Law , $f(i+1) = \frac{c^2}{i+1}$ and $f(i) = \frac{c^2}{i}$. So,

$$L = \sum_{i=1}^{8}\left(\sqrt{1 + c^4\left(\frac{1}{i^2+i}\right)^2}\right)$$

We know, the total arc length, L' of y = f(x) will be

$$\int_a^b \sqrt{1 + \left(\frac{d}{dx}(f(x))\right)^2}\,dx$$

where a, and b are the extremum on x – axis.
Here, $f(x) = \frac{c^2}{x}$. So,

$$L' = \int_1^9\left(\sqrt{1 + \frac{c^4}{x^4}}\right)dx = \left[\frac{x\left(\sqrt{\frac{c^4}{x^4}+1}\right)\left(2F_1\left(\frac{-1}{2}, \frac{-1}{4}; \frac{3}{4}; -\frac{x^4}{c^4}\right)\right)}{\sqrt{\frac{x^4}{c^4}+1}}\right]_1^9$$

$$L' = -\frac{c^2}{9}\left(2F_1\left(\frac{-1}{2}, \frac{-1}{4}; \frac{3}{4}; -\frac{6561}{c^4}\right)\right) - 9\left(2F_1\left(\frac{-1}{2}, \frac{-1}{4}; \frac{3}{4}; -\frac{1}{c^4}\right)\right)$$

where, 2F1(a, b;c;x) is the hypergeometric function(5).
Equating, we get,

$$L + \varepsilon = L'$$

$$\Rightarrow \sum_{i=1}^{8}\left(\sqrt{1 + c^4\left(\frac{1}{i^2+i}\right)^2}\right) + \varepsilon = \int_1^9\left(\sqrt{1 + \frac{c^4}{x^4}}\right)dx$$

where, ε= degree of error.
Thus, $\varepsilon = abs\left(\int_1^9\left(\sqrt{1 + \frac{c^4}{x^4}}\right)dx - \sum_{i=1}^{8}\left(\sqrt{1 + c^4\left(\frac{1}{i^2+i}\right)^2}\right)\right)$ where abs(.) denotes the absolute value of a given function.

So, the absolute percentage error is:

$$\varepsilon' = \frac{abs\left(\int_1^9\left(\sqrt{1+\frac{c^4}{x^4}}\right)dx - \sum_{i=1}^{8}\left(\sqrt{1+c^4\left(\frac{1}{i^2+i}\right)^2}\right)\right)}{\int_1^9\left(\sqrt{1+\frac{c^4}{x^4}}\right)dx} \times 100$$

where, c^2 = Frequency of digit 1 as the first digit.

(5) It is a special type of function which is veritably the solution of a second-order linear differential equation.
2F1a, b;c;x=i=0aibicixii!
ti={1 i=0 tt+1t+2t+i-1 i>0 is the rising Pochhammer symbol.

4. Validation of the Stratagem

In this paper we have proposed an empirical formula named as Arc-Length Estimate Conjecture[17]. This formula has many advantages over some well-known data validation laws such as Benford's law, Zipf's law, Lotka's law, etc. This formula is basically derived from Zipf's law and it deals with the approximation of Arc-Length of the Zipfian distribution.
The datasets are concentration of :-

- I131(Iodine-131)
- Cs134(Caesium-134)

L and L' are computed individually for each dataset i.e., concentration levels of I131 and Cs134, then we will calculate a bound for error, up to which the deviations in datasets are permissible. There is a scale defined for each dataset by Arc-Length Estimate Conjecture which sets a limit of error for that specific dataset. If the error of that dataset is within the specified error-range then we can declare the dataset as theoretically valid.

4.1 I131 (Iodine - 131) Analysis

We will validate the dataset of the concentration of I131 (Iodine 131)[18] using Arc-Length Estimate Conjecture as aerosol particles which were measured in some specific location in Europe. Here the concentration of the chemical is measured in (Bq/m3) where Bq=Radon levels.
We considered the first non-zero digit after the decimal of the concentration levels.
Here, c2 is defined as the frequency of occurrence of the digit 1 as the first digit.
So, as we have calculated before, for I131 (Iodine-131), c^2 =502.
We know that $L = \sum_{i=1}^{8} \left(\sqrt{1 + c^4 \left(\frac{1}{i^2+i} \right)^2} \right)$

Let, $L_i = \left(\sqrt{1 + c^4 \left(\frac{1}{i^2+i} \right)^2} \right) \quad \forall\, i \in \{1, 2, 3..., 9\}$

Table 2. Tabular representation of corresponding values of Li s
Value of 'i'

Value of 'i'	*Value of 'L_i'*
1	251.00199
2	83.67264
3	41.84528
4	25.11991
5	16.76319
6	11.99414
7	9.01989
8	7.04357

So, $L = \sum_{i=1}^{8}\left(\sqrt{1 + c^4\left(\frac{1}{i^2+i}\right)^2}\right) = 446.4606191$

Now, we will calculate $L' = \int_1^9\left(\sqrt{1 + \frac{c^4}{x^4}}\right)dx = \left[\frac{x\left(\sqrt{\frac{c^4}{x^4}+1}\right)\left(2F_1\left(\frac{-1}{2}, \frac{-1}{4}; \frac{3}{4}; -\frac{x^4}{c^4}\right)\right)}{\sqrt{\frac{x^4}{c^4}+1}}\right]_1^9$

Thus,

$L' = -\frac{c^2}{9}\left(2F_1\left(\frac{-1}{2}, \frac{-1}{4}; \frac{3}{4}; -\frac{6561}{c^4}\right)\right) - 9\left(2F_1\left(\frac{-1}{2}, \frac{-1}{4}; \frac{3}{4}; -\frac{1}{c^4}\right)\right)$

where, $2F_1(a, b; c; x)$ is the hypergeometric function.

So, $L' = \int_1^9\left(\sqrt{1 + \frac{502^2}{x^4}}\right)dx = \left[\frac{x\left(\sqrt{\frac{502\times502}{x^4}+1}\right)\left(2F_1\left(\frac{-1}{2}, \frac{-1}{4}; \frac{3}{4}; -\frac{x^4}{502\times502}\right)\right)}{\sqrt{\frac{x^4}{502\times502}+1}}\right]_1^9 \approx 446.463$

Now, to find the error bound we have to calculate the absolute percentage error :-

$$\varepsilon' = \frac{abs\left(\int_1^9\left(\sqrt{1+\frac{c^4}{x^4}}\right)dx - \sum_{i=1}^{8}\left(\sqrt{1+c^4\left(\frac{1}{i^2+i}\right)^2}\right)\right)}{\int_1^9\left(\sqrt{1+\frac{c^4}{x^4}}\right)dx} \times 100$$

Plugging values,

$$\varepsilon' = \frac{abs(446.463 - 446.4606191)}{446.463} \times 100 = 0.000533275\%$$

∴ The absolute percentage error is $\varepsilon' = 0.000533275\%$

This error range gives us an estimate that how much the set of data is deviating from the ideal behavior. In this case, the data is deviating 0.000533275% from the ideal assigned value.

4.2 Cs134 (Cesium - 134) Analysis

We will validate the dataset of the concentration of Cs^{134} (Cesium 134)[19] using Arc-Length Estimate Conjecture. Here the concentration of the chemical is measured in (Bq/m^3) where Bq=Radon levels.

We considered the first non-zero digit after the decimal of the concentration levels.

Here, c^2 is defined as the frequency of occurrence of the digit 1 as the first digit.

So, as we have calculated before, for Cs^{134} (Cesium-134), $c^2 = 369$.

We know that $L = \sum_{i=1}^{8}\left(\sqrt{1 + c^4\left(\frac{1}{i^2+i}\right)^2}\right)$

Let, $L_i = \left(\sqrt{1 + c^4\left(\frac{1}{i^2+i}\right)^2}\right) \quad \forall\, i \in \{1, 2, 3..., 9\}$

Table 3. Tabular representation of corresponding values of L_i

Value of 'i'	*Value of 'L_i'*
1	184.50271
2	61.50813
3	30.76626
4	18.47708
5	12.34058
6	8.84244
7	6.66473
8	5.22165

So, $L = \sum_{i=1}^{8}\left(\sqrt{1 + c^4\left(\frac{1}{i^2+i}\right)^2}\right) = 328.3235851$

Now, we will calculate $L' = \int_1^9\left(\sqrt{1 + \frac{c^4}{x^4}}\right)dx = \left[\frac{x\left(\sqrt{\frac{c^4}{x^4}+1}\right)\left(2F_1\left(\frac{-1}{2}, \frac{-1}{4}; \frac{3}{4}; -\frac{x^4}{c^4}\right)\right)}{\sqrt{\frac{x^4}{c^4}+1}}\right]_1^9$

Thus,

$L' = -\frac{c^2}{9}\left(2F_1\left(\frac{-1}{2}, \frac{-1}{4}; \frac{3}{4}; -\frac{6561}{c^4}\right)\right) - 9\left(2F_1\left(\frac{-1}{2}, \frac{-1}{4}; \frac{3}{4}; -\frac{1}{c^4}\right)\right)$

where, $2F_1(a, b; c; x)$ is the hypergeometric function.

So, $L' = \int_1^9\left(\sqrt{1 + \frac{369^2}{x^4}}\right)dx = \left[\frac{x\left(\sqrt{\frac{369\times369}{x^4}+1}\right)\left(2F_1\left(\frac{-1}{2}, \frac{-1}{4}; \frac{3}{4}; -\frac{x^4}{369\times369}\right)\right)}{\sqrt{\frac{x^4}{369\times369}+1}}\right]_1^9 = 328.327$

Now, to find the error bound we have to calculate the absolute percentage error :-

$\varepsilon' = \frac{abs\left(\int_1^9\left(\sqrt{1+\frac{c^4}{x^4}}\right)dx - \sum_{i=1}^{8}\left(\sqrt{1+c^4\left(\frac{1}{i^2+i}\right)^2}\right)\right)}{\int_1^9\left(\sqrt{1+\frac{c^4}{x^4}}\right)dx} \times 100$

Plugging values,

$\varepsilon' = \frac{abs(328.327-328.3235851)}{328.327} \times 100 = 0.001040076\%$

∴ The absolute percentage error is $\varepsilon' = 0.001040076\%$

This error range gives us an estimate that how much the set of data is deviating from the ideal behavior. In this case, the data is deviating 0.001040076% from the ideal assigned value.

5. Comparative Analysis with Zipf's Law

In the section we will to verify the accuracy and correctness of the dataset containing the concentration of I^{131} (Iodine – 131) and Cs^{134} (Cesium – 134) as aerosol particles which were measured at different locations across Europe by effectively using Zipf's Law. Several parts of Europe were adversely affected during the nuclear cataclysm and the real impact of this catastrophic event is still a controversial topic across the globe. So, in this section we will try to verify the severity of this event to utmost accuracy.
The datasets we are using are taken are available online on Kaggle.
The datasets are concentration of :-

- I^{131}(Iodine-131)
- Cs^{134}(Caesium-134)

Note: A log-log graph (Rank is denoted by the x-axis and the frequency is represented by the y-axis) is used to show an enhanced and detailed analysis of the extremities. If the log-log plot closely resembles a straight line then the dataset is more prone to follow the Zipf's law. If the plot deviates from a straight-line representation, then the dataset doesn't tend to follow the Zipf's law.

5.1 I^{131} (Iodine - 131) Analysis

We will validate the dataset of the concentration of I^{131} (Iodine - 131) as aerosol particles which were measured in some specific location in Europe. Here the concentration of the chemical is measured in (Bq/m^3) where Bq=Radon levels.
We considered the first non-zero digit after the decimal of the concentration levels.

Table 4. Digit-wise frequency of the first non-zero digit of the concentration of I131 (Iodine – 131) of Chernobyl nuclear disaster.

Digit	*Frequency*
1	502
2	338
3	212
4	164
5	158
6	115
7	94
8	70
9	82

Adding up all the frequency we get $\sum f_i = 1735$.

Here, basically the first digit of the concentration levels of I^{131} at different places in Europe after the Chernobyl event is calculated. The frequency denotes the number of times digit 'k' has occurred as the first digit where $k \in \{1, 2, ..., 9\}$.
Now, we will check the validity of the I^{131} (Iodine – 131) dataset using Zipf's law.

Table 5. Rank-Frequency table for Zipf's law (Product = Rank × Frequency).

Digit	*Frequency*	*Rank*	*Product*
1	502	1	502
2	338	2	676
3	212	3	636
4	164	4	656
5	158	5	790
6	115	6	690
7	94	7	658
8	70	8	560
9	82	9	738

Now, we will plot the log-log graph for the I^{131} (Iodine – 131) concentration dataset where Rank is represented by the x-axis and frequency is represented by the y-axis.

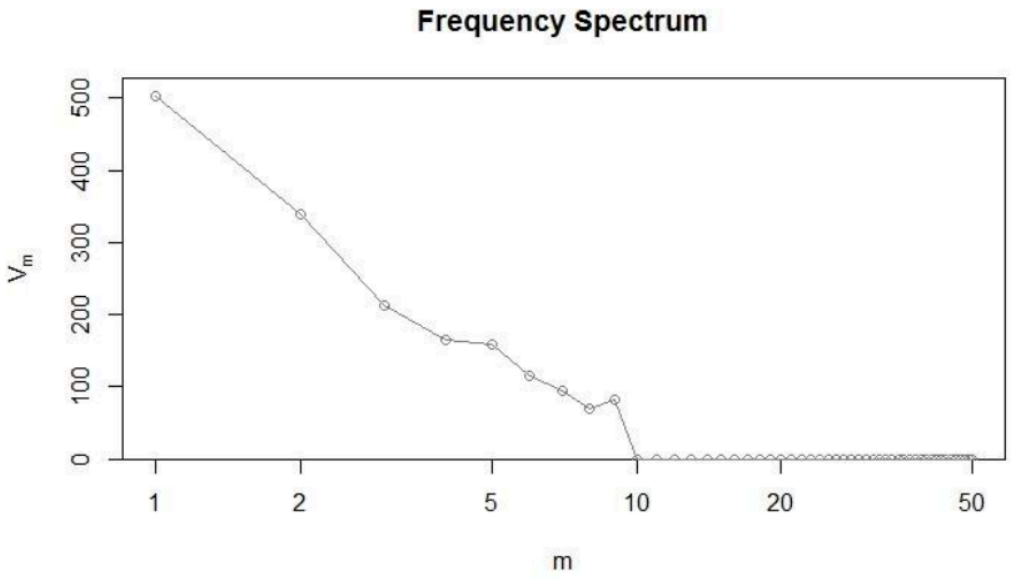

Fig. 2. Log-log graph of Zipf's law of I^{131} (Iodine – 131) concentration dataset.

The scattered curve in the above log-log plot closely resembles a straight line with negative slope. Thus, the I^{131} (Iodine – 131) concentration dataset follows Zipf's law.
Hence, from the above analysis by Zipf's law we can say that the I^{131} (Iodine – 131) concentration (Bq/m^3) dataset of Chernobyl nuclear blast is valid.

5.2 Cs^{134} (Cesium - 134) Analysis

We will validate the dataset of the concentration of Cs^{134} (Cesium 134) as aerosol particles which were measured in some specific location in Europe. Here the concentration of the chemical is measured in (Bq/m^3) where Bq=Radon levels.
We considered the first non-zero digit after the decimal of the concentration levels.

Table 6. Digit-wise frequency of the first non-zero digit of the concentration of Cs^{134} (Cesium – 134) of Chernobyl nuclear disaster.

Digit	*Frequency*
1	369
2	208
3	169
4	128
5	97
6	85
7	98
8	55
9	65

Adding up all the frequency we get $\sum f_i = 1274$.

Here, basically the first digit of the concentration levels of I^{131} at different places in Europe after the Chernobyl event is calculated. The frequency denotes the number of times digit 'k' has occurred as the first digit where $k \in \{1, 2, ..., 9\}$.

Now, we will check the validity of the Cs^{134} (Cesium – 134) dataset using Zipf's law.

Table 7. Rank-Frequency table for Zipf's law (Product = Rank×Frequency).

Digit	*Frequency*	*Rank*	*Product*
1	369	1	369
2	208	2	416
3	169	3	507
4	128	4	512
5	97	5	485
6	85	6	510
7	98	7	686
8	55	8	440
9	65	9	585

Now, we will plot the log-log graph for the Cs^{131} (Cesium – 134) concentration dataset where Rank is represented by the x-axis and frequency is represented by the y-axis.

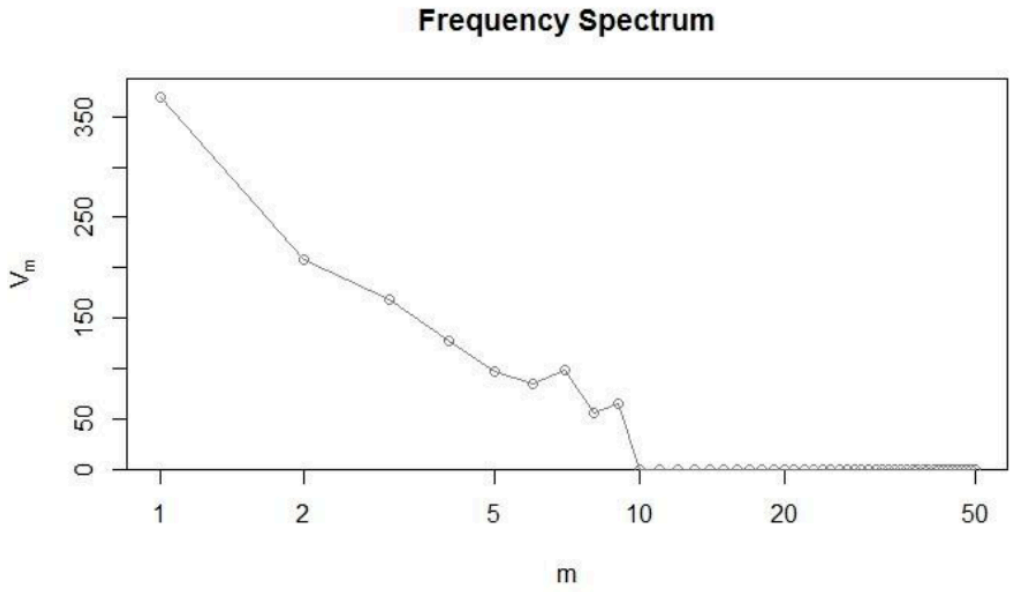

Fig. 3. Log-log graph of Zipf's law of Cs^{134} (Cesium – 134) concentration dataset.

The scattered curve in the above log-log plot closely resembles a straight line with negative slope. Thus, the Cs^{134} (Cesium 134) concentration dataset follows Zipf's law.
Hence, from the above analysis by Zipf's law we can say that the Cs^{134} (Cesium 134) concentration (Bq/m^3) dataset of Chernobyl nuclear blast is valid.

6. Conclusion

The Chernobyl Tragedy was one the deepest scars, that wrath of science has put into the face of humanity and mankind. Though till date, we don't have complete control on nuclear forms of energy, still from the CNPP incident, we should be aware that a tiny disruption of any radioactive elements from its proposed state can pose a huge threat to lives.

There are certain advantages of Arc-Estimate Conjecture over Zipf's law. In Zipf's law there isn't conformation that whether a dataset is valid or not because in Zipfian distribution there is no estimate for conformation range under which we can declare a dataset as theoretically valid. Zipf's law doesn't have any error bounds, which would help us to know how much deviation of the dataset from ideal case is acceptable. Also, Arc- Length Estimate Conjecture meets the requirements of the above mentioned drawbacks of Zipf's law.

There are some important observations that we can draw from the above validation results. First, the absolute percentage error for both the datasets are the same up to four decimal places. Next, from the above observation we may make a hypothesis that the absolute percentage error is independent of the term c^2 i.e., the percentage error will be bounded by a specific conformity range provided that the dataset is valid.

There are many future scope of research on Arc-Length Estimate Conjecture. Like, a conformity range for absolute percentage error can be calculated for this conjecture, so that if the absolute percentage error of a specific dataset falls inside this range, then we can declare it as theoretically valid. In this paper it is not proven that the value of

absolute percentage error doesn't depend on the term c^2. In future it may be proven that the adjacent statement is true and further research can be done on this conjecture.

RESULTS

There are certain advantages of our Notion in comparison with Zipf's law :-

- In Zipf's law there isn't conformation that whether a dataset is valid or not because in Zipfian distribution there is no estimate for conformation range under which we can declare a dataset as theoretically valid.
- Zipf's law doesn't have any error bounds, which would help us to know how much deviation of the dataset from ideal case is acceptable.
- Arc- Length Estimate Conjecture meets the requirements of the abovementioned drawbacks of Zipf's law.

Some observations that we can draw from the above validation are :-

- The absolute percentage error for both the datasets are same up to four decimal places.
- From the above observation we may make a hypothesis that the absolute percentage error is independent of the term c2 i.e., the percentage error will be bounded by a specific conformity range provided that the dataset is valid.

Future scope of research on the stratagem :-

- A conformity range for absolute percentage error can be calculated for this conjecture, so that if the absolute percentage error of a specific dataset falls inside this range, then we can declare it as theoretically valid.
- In this paper it is not proven that the value of absolute percentage error doesn't depend on the term c2. In future it may be proven that the adjacent statement is true and further research can be done on this conjecture.

References

1. Paraskevoulakos C., Forna-Kreutzer J. and others (2021), "Investigating the microstructure and mechanical behavior of simulant "lava-like" fuel containing materials from the Chernobyl reactor unit 4 meltdown", Materials & Design, Elsevier. Vol. 201. 109502
2. D'Alessandro A. (2020), "Benford's law and metabolomics: A tale of numbers and blood", Transfusion and Apheresis Science. Elsevier. Vol. 59. 103019
3. Hill, T. (2001), "A Statistical Derivation of the Significant-Digit Law. Institute of Mathematical Statistics. Statistical Science", Vol. 10. pp. 354-363
4. Nigrini M. and Drake P. (2000), "Computer assisted analytical procedures using Benford's Law", Journal of Accounting Education. Vol. 18. pp. 127-149
5. Newcomb, S. (1881), "Note on the Frequency of Use of the Different Digits in Natural" . American Journal of Mathematics. Vol. 4. pp. 39-40
6. Horton, J. and Krishna K. and Wood, A. (2020), "Detecting academic fraud using Benford law: The case of Professor James Hunton" Research Policy
7. Grishanin E. (2009), "The Role of Chemical Reactions in the Chernobyl Accident", Physics of Atomic Nuclei, Springer. Vol 73, pp. 2296-2300.
8. Saenko V. and others (2011), "The Chernobyl Accident and its Consequences", Clinical Oncology, Elsevier. Vol 23, pp. 234-243.
9. Moros, M., Andersen, T.J., Schulz-Bull, D., H ¨ausler, K., Bunke, D., Snowball, I., Kotilainen, A., Zill ´en, L., Jensen, J.B., Kabel, K., Hand, I., Leipe, T., Lougheed, B.C., Wagner, B., Arz, H.W., 2017. Towards an event stratigraphy for Baltic Sea sediments deposited since AD 1900: approaches and challenges. Boreas 46,

129–142.
10. Neumann, Peter G, "Statistical metalinguistics and Zipf/Pareto/Mandelbrot", Springer, Berlin, 2011.
11. Ik ¨aheimonen, T.K., Outola, I., Vartti, V.-P., Kotilainen, P., 2009. Radioactivity in the Baltic Sea: inventories and temporal trends of 137Cs and 90Sr in water and sediments. J. Radioanal. Nucl. Chem. Vol. 282 (2), 419–425.
12. Herrman, J., 2000. Levels of radioactivity. In: Nielsen, S.P. (Ed.), The radiological exposure of the population of the European Community to radioactivity in the Baltic Sea. Radiation Protection, Vol. 110. European Commission, Luxembourg, ISBN 92-828-7864-3, pp. 77–129.
13. Holby, O., Evans, S., 1996. The vertical distribution of Chernobyl-derived radionuclidesin a Baltic Sea sediment. J. Environ. Radioact. Vol. 33, 129–145.
14. Ojala, A.E.K., Luoto, T.P., Virtasalo, J.J., 2017. Establishing a high-resolution surface sediment chronology with multiple dating methods – testing 137Cs determination with Nurmij ¨arvi clastic-biogenic varves. Quat. Geochronol. Vol. 37 (32–41), 2017.
15. Yoshimura, K., Onda, Y., Fukushima, T., 2014. Sediment particle size and initial radiocesium accumulation in ponds following the Fukushima DNPP accident. Sci. Rep. 4, 4514.
16. Yu, C., Virtasalo, J.J., Karlsson, T., Peltola, P., ¨Osterholm, P., Burton, E.D., Arppe, L., Hogmalm, J.K., Ojala, A.E.K., Åstr ¨om, M.E., 2015. Iron behavior in a northernestuary: large pools of non-sulfidized Fe(II) associated with organic matter. Chem. Geol. Vol. 413, pp. 73–85.
17. Salminen-Paatero, S., Th ¨olix, L., Kivi, R., et al., 2019. Nuclear contamination sources in surface air of Finnish Lapland in 1965–2011 studied by means of 137Cs, 90Sr, and total beta activity. Environ. Sci. Pollut. Res. Vol. 26, pp. 21511–21523.
18. Sahla, M., Turkia, T., Nieminen, A., R ¨as ¨anen, T., Haapam ¨aki, J., ja Hoikkala, J., Suominen, F., Kantanen, J., 2020. Ilmakuvakartoitus ihmispaineista Suomen rannikon merialueilla (in Finnish). Meriluonnonsuojelu, Luontopalvelut, Mets ¨ahallitus. The Data set is viewable in the VELMU map service. Visited 1.3.2021.

Gentrification and Policing of Minorities: fMRI-Tested Inevitability of Racial Bias Demands a Policy Pivot

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Article Synopsis

Gentrification is a growing struggle in America. It is accompanied by changing social dynamics which especially induces pressures on lower socioeconomic status (SES) Americans. One dynamic under scrutiny is the changes in policing patterns of gentrifying regions throughout neighborhoods and public schools. These domains experience disproportionate policing, resulting in increased punishment and even incarceration. Despite racial bias training, fMRI data suggest that implicit biases are difficult to overcome. Therefore, limiting police's opportunities to act upon biases via limiting gentrification could more significantly mitigate its consequences for lower SES individuals.

Gentrification and Policing of Minorities: fMRI-Tested Inevitability of Racial Bias Demands a Policy Pivot

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Abstract

Mental illness affects all populations but has disproportionately affected college students in the recent past. Universities provide a unique setting in which students often live away from their families and must make an effort to stay socially engaged. While plenty of research on mental health has been conducted on children and adults, the field is lacking in research on college-aged individuals. The present study identifies the frequency of symptoms of depression and anxiety among the Duke University undergraduate community, as well as demographic factors that are correlated with these symptoms. The data were obtained through a survey asking students about their experiences with well-established symptoms of Major Depressive Disorder and Generalized Anxiety Disorder. The data indicate that students at Duke University experience symptoms of depression and anxiety at a higher rate compared to the average 18-29 year old person in the United States. Other findings include a greater prevalence of mental illness symptoms among low-income students, students with small social-circles, and non-religious students. The results of this study and related studies to follow will be used to inform improvements to mental health counseling and outreach to at-risk individuals at universities across the United States.

Introduction

Gentrification increasingly challenges metropolitan America. As demand for housing becomes more competitive and costs increase, racial minorities are disproportionately displaced from their homes. During this process, minority families may be subject to the consequences of racial bias in the form of over policing within schools and neighborhoods. Visual-stimulus fMRI data in the amygdala suggest that such racial bias may be implicit, which may also problematically mold law enforcement dynamics in gentrifying regions. Therefore, gentrification could amplify opportunities for racial bias in the form of unfair policing. While police undergo racial bias training, its innate nature suggests that anti-gentrification policy interventions may better mitigate the integrated effects of racial bias, gentrification, and over policing.

Gentrification is the Axis of Housing, Public Schooling, and Racial Bias

The combination of economic and social pressures causes shifts in population patterns. With American population growth on an exponential rise, economic pressures will become more significant as rents increase in response to demand. Influxes of businesses and wealthier residents may contribute to increased housing costs (Wharton, 2008). Unfortunately, many residents cannot sustain such costs and are thus forced to relocate in a process known as gentrification.

Gentrification is the axis of America's housing, schooling, and racial bias issues. Many gentrifying

areas in America see movement of races in different directions; the influx of whites frequently displaces formerly black neighborhoods with resources pouring in only after their exodus (Kirkland, 2008). Gentrification perpetuates the opportunity for authorities to exercise racial bias, particularly in schools and neighborhoods.

As gentrifying neighborhoods undergo demographic changes, so do its public schools; wealthier, gentrifying areas have more property tax to support their schools while displaced residents must relocate to poorly-resourced school districts. Notably, underdeveloped schools with high minority populations are often targets for school resource officers (SROs) to excessively enforce zero tolerance policies (ZTPs), which disproportionately disciplines minority students and accentuates the school-to-prison pipeline (Stpp). Concurrently, enhanced law enforcement to oversee and facilitate demographic changes during gentrification also contribute to disproportionate discipline in the neighborhood. Police adopt Order Maintenance Policing (OMP) strategies, allowing them to use flawed personal judgement to crack down on suspected violence, targeting minority populations.

In this paper, we will first discuss how fMRI amygdala expression in response to race using facial cues can record implicit racial bias. Second, we will observe the specific impacts of gentrification in the context of how over policing by SROs in public schools of gentrifying areas use ZTPs to act upon racial bias and expand the Stpp. Third, we will observe how over policing in gentrifying neighborhoods via OMP strategies also present opportunities to act upon racial bias. Throughout our discussion, we will cross-apply our findings from fMRI data to analyze the behavioral consequences within each domain. Finally, we will suggest policy approaches against the consequences of gentrification and racial bias: whether we should target gentrification or racial bias.

fMRI Evidence Demonstrates the Innate Nature of Implicit Racial Bias

fMRI brain scans of the amygdala were used to identify implicit racial bias. Activity in the amygdala is suggested to reflect threat detection and the fear response, which we employ to indicate implicit racial bias (Ronquillo et al., 2007). Wheeler and Fiske (2005) presented white participants with pictures of white (in-group) and black (out-group) faces and asked them to perform a series of social and non-social tasks (Wheeler and Fiske, 2005). They found significantly greater left amygdala activity for both tasks when participants were presented with black faces, suggesting an association between the black race and threats. Indeed, multiple studies have confirmed that differential amygdala firing in response to skin-tone does occur, confirming the associations between racial bias and perceived fear or threat (Ronquillo et al., 2007). No differences were found in non-social tasks, suggesting that implicit racial bias may only play a significant role in social interactions and the formation of stereotypes. These findings may indicate the innate nature of racial bias, as the amygdala modulates the fear and threat response.

Other relevant research confirms that racial bias is an innate trait related to cognitive processes, especially emotion, that exists across multiple cultures. Shen et al. (2018) concluded from fMRI data that participants tend to show more empathy and greater activity in the anterior cingulate cortices (ACC) towards the in-group as compared to the out-group (Shen et al., 2018). These studies agree with Wheeler and Fiske's findings about the role of cognitive functions, particularly emotion, in implicit racial bias, and demonstrate that implicit racial bias can arise during social interactions.

Neurocognitive data suggesting an innate nature of racial bias will guide the evaluation of policy interventions to prevent the School-to-Prison Pipeline (Stpp) in public schools and over policing in neighborhoods of gentrifying areas.

The Role of Gentrification in the Expansion of the School-to-Prison Pipeline in Public Schools

In public schools, racial prejudice plays a role in feeding minority students into the School-to-Prison Pipeline (Stpp), a phenomenon where disproportionate discipline by SROs subject minorities to face legal trouble at a young age, increasing dropouts and the chance of committing crimes. Indeed, racial minorities are underrepresented in school populations, but are overrepresented in student crime; those students are more likely to be punished by School Resource Officers (SROs) and in court by judges, and thus face obstacles in their educational careers (Redfield and Nance, 2016). Compared to white students, students of African American origin are from 2.19 to 3.78 times more likely to be referred by SROs for behavioral problems (Russell et al., 2011). Additionally, black

students tend to be subject to more severe punishments than white students for the same offense (Nicholson-Crotty et al., 2009). The socioeconomic gap between white and black families is also shown to be positively correlated with the difference between the number of white and black referrals in the juvenile court and that between the number of exclusions of black and white students due to disciplinary reasons (Nicholson-Crotty et al., 2009). Therefore, as wealthy gentrifiers move into an area with mostly racial minorities, more students of racial minorities will be excluded from school and sent to jail.

Gentrification amplifies the Stpp by influencing massive changes in local demographics. As rising costs and property taxes in gentrifying regions price out families of lower socioeconomic status, they exceedingly become pushed out of their neighborhoods (Zukin, 2009). As a result, the movement of wealthier families into an area transforms districts from poor to expensive areas (Zukin, 2009). This 'pricing-out' effect of gentrification segregates districts and thus the public schools associated with them; gentrified families become so geographically isolated from schools in wealthier areas that they can no longer pursue attendance in middle-class schools (Rothstein, 2015). The segregation of neighborhood and public school populations is compounded by the effects of property taxes; because local funding is tied to property values, gentrified families leave students in schools with poor resources while the gentrifiers 'lock away' higher quality education (Ireland, 2003). These factors combine to create a direct link between gentrification and the Stpp. Gentrifying regions see an increase in police presence in public schools, and the higher concentration of minorities due to relocation creates 'hot spots' for police to criminalize behaviors that are more common to minorities, thus amplifying the Stpp (Leguichard, 2019). These 'hot spots' allow SROs to use multiple mechanisms to punish minority students.

One mechanism for enhancing the Stpp is manipulating the SROs' vague authority to punish students. White Americans tend to have higher socioeconomic status and greater academic performance in school, which can lead to a lower likelihood of being expelled for disciplinary reasons (Rocque and Snellings, 2018). However, racial discrimination also plays a large role. When SROs' roles are not properly established and communicated by SRO programs, they tend to become involved in student discipline because they believe that disobedient students of African American origin are "unsalvageable" and likely to end up in jail (Rocque and Snelling, 2018; Finn et al., 2005). SRO officers, 69 percent of whom are white, may thus naturally show less empathy towards students of color, members of their out-group, than white students, members of their in-group (Shen et al., 2018; Education Week Research Center, 2018). The introduction of more police presence as a result of gentrification will only amplify the negative attitude SROs have towards the "unsalvageable" students (Leguichard, 2019). As racial bias is directly linked to brain parts that manage emotions and cognition, it may be difficult for SROs to overcome implicit biases and over-discipline minority students a preventative, risk-reducing measure.

Another mechanism of amplifying the Stpp that is highly influenced by gentrification is the enforcement of zero tolerance policies (ZTPs). SROs in public schools in gentrified regions are more likely to incorporate punitive measures such as ZTPs in response to disciplinary problems (Welch and Payne, 2010). The creation of ZTPs, combined with the 'hot spot' policing in these schools, allows SROs to punish more students. The direct result is an expanded school-to-prison pipeline that makes minority students more likely to drop out of or fail high school, be involved in crimes, and have less opportunity to be employed in the future (Fabelo et al., 2011; Kupchik, 2009; Rocque and Snelling, 2018).

Overall, implicit racial bias, the failure of schools to effectively communicate their roles, selective application of ZTPs, and gentrification creates opportunities for SROs to feed students of racial minorities into the criminal justice system. It follows that schools should enhance SRO training to prevent implicit racial bias incidents; however, this may be difficult due to the innate nature of racial bias. Although many studies show that racial bias training leads to reduced bias in the short term, they do not suggest that this effect can last in the long term (Devine et al., 2012). Alternatively, a decrease in gentrification can subsequently reduce the influx of white SROs into predominantly colored schools, which would eliminate the opportunity for police to profile students in the

first place. These propositions in tandem may greatly mitigate the flow of the Stpp.

The Role of Gentrification in the Over Policing of Neighborhoods

Gentrification also has interesting implications for the role of police presence. Social dynamic changes can lead to a change in the number of police present in an area or the police's aggression in enforcing laws. The latter is a trend seen across gentrifying neighborhoods in America (Laniyonu, 2018). A leading theory for the positive correlation between gentrification and aggression in policing is the 'postindustrial policing hypothesis,' which argues that patterns of policing aggressiveness are results of top-level municipal strategies that aim to bring in middle to upper class white families with education (Laniyonu, 2018; Zuk, 2015). Thus, not only does gentrification drive the influx of white families while pushing socioeconomic minorities out, but it also causes shifts in attitudes of policing to amplify the relocation process.

Under the guise of 'revitalization,' municipal authorities across the country drive the influx of families of higher socioeconomic status, typically at the cost of minorities - a direct snapshot of gentrification-promoting policies. As a result, policing is widely used as a tool in the revitalization process. Therefore, gentrification changes policing patterns in a fashion where it increases aggression in order to accelerate gentrification: a positive feedback loop (Laniyonu, 2018).

Specifically, gentrification causes changes in policing by changing municipal policing strategies. Municipal police departments deliberately prepare parts of the city for gentrification or inherently transition to aggressive policing as a result of the socioeconomic change gentrification produces (Sharp, 2013). These overaggressive practices are designed to cleanse the neighborhood from poor perceptions, by addressing fears of crime, perceptions of social disorder, and behavior disruptive to "regular residents" (Sharp, 2013). Such behavior is a proxy used by police departments to enhance gentrification. Specifically, those overaggressive practices are maintained by policies to maintain 'quality of life,' formally known as a strategy called Order Maintenance Policing (OMP) (Laniyonu, 2018). OMP enables local police to transition away from prioritizing serious offenses and upholding detachment between police and bureaucracy; instead, the OMP strategy directs police to increase aggression, overpoliced petty offenses, and take preventative measures to inhibit "disorder" (Laniyonu, 2018). The long-term result of the OMP approach is a dissipation in trust with police, increased punitive measures for low-level offenses, and decreased social tolerance - all for the sake of social control under the guise of creating an image of high 'quality of life' (Vitale, 2008). Bluntly, the OMP strategy gives police carte blanche to police based on racial biases, which is exactly how law enforcement becomes alienated from the communities they are meant to serve.

Police response to gentrifier demands also amplifies over policing. As social dynamics change, the police feel a sense of peer pressure to crack down on suspicious activity reported by gentrifiers (Laniyonu, 2018). A greater demand for policing from gentrifiers leads the police to believe that there is more suspicious activity, resulting in more investigation and more arrests, amplifying over policing. This phenomenon can be explained with fMRI data. Peer influence can result in greater connectivity between the anterior insular cortex (AIC) and the amygdala, the hippocampus, and the ventromedial prefrontal cortex (vmPFC), brain regions that regulate emotions and cognition (Sherman et al., 2019). The presentation of peer opinions can also influence people's views on a certain issue, making them more likely to conform to these opinions (Wake et al., 2019). Conformity to peers is positively correlated with activity in the posterior medial frontal cortex (pMFC), the lateral superior temporal gyrus (STG), and the posterior cingulate cortex (PCC), which are brain areas associated with cognition (Wake et al., 2019). It can be concluded that the influence of peer pressure is greatly associated with brain areas involved in cognition, and that conformity to peer influence is natural and innate. Racial bias may be a consequential form of such peer pressure.

The effect of peer pressure on over policing can be demonstrated by examining the rate and location of 311 calls - a subsidiary of 911 calls for community disagreements or issues. Empirically, 311 calls in New York for offenses within the scope of the OMP strategy were significantly higher from gentrifying areas along with crime rates, suggesting that over policing was directly influenced by citizen demand, confirming

previous findings (Laniyonu, 2018). Furthermore, to solidify the causality of citizen demand on police behavior, observing the rates of policing before and after the influx of gentrifiers will establish reverse causality; that is, the arrival of gentrifiers should increase the previous level of policing. Indeed, a five-year study on the NYPD found that police summons and arrests increased threefold in low socioeconomic status communities after a large influx of white residents with police responding to the complaints 92.5 percent of the time (Community Service Society, 2019). Thus, gentrification directly increases over policing with citizen demands of gentrifiers as the vehicle for aggression.

Overall, policing in neighborhoods seems to read and react to the predominant demographic in residents. As gentrification changes social dynamics, police departments increase their aggression in enforcement of the law by both shifting to the OMP policing strategy and accommodating citizen demands of gentrifiers; the result is a positive feedback loop where gentrification is accelerated. Both of these approaches to increasing aggression are dangerously prone to racial bias. The OMP strategy seems to dismantle neutrality that police training is meant to achieve; this allows suspicion to be enough to take action. Gentrifier demands for controlling perceived threats and suspicious activity encourages more police presence via 311 calls that are not only almost always responded to by police, but also significantly increase arrests of minorities. Because of the difficulty for the police to restrain their urge to conform to gentrifier opinions and to maintain neutrality, as suggested by fMRI data and evidence regarding racial bias training, gentrification must be reduced in order to reliably lower over policing in neighborhoods.

Conclusion and Suggestions for Future Research

As gentrification is rapidly accelerating, policymakers must acknowledge its growing consequences in public schools and neighborhoods. An influx of white families with high socioeconomic status into an area inhabited by mostly racial minorities can lead to an increase in the number of white SROs and gentrifiers who demand aggressive policing towards the minorities. Combined with implicit racial bias among the white gentrifiers, gentrification can result in the creation of ZTPs by SROs, which send more minority students into the Stpp, increasing high school dropout rates and the chance of future criminal activity. In the context of neighborhoods, gentrification is connected with the use of more aggressive policing tactics, significantly increasing the rate of incarceration of residents. Although racial bias training may help reduce Stpp and over policing, it may not be reliable because of the innate nature of implicit racial bias and the use of the OMP strategy. Instead, we suggest that future research should aim to evaluate policies that reduce gentrification, which cuts out the source of implicit racial bias. For example, an experimental proposal in New York to mitigate gentrification is the implementation of zoning regulations that taper the difficulty for displacement across the city combined with redevelopment of areas on the verge of vacancies to prevent new gentrifiers (Marcuse, 1985; Rose, 2002). Attacking gentrification at its root is likely the single most promising approach to eliminating the infamous axis of housing, schooling, and racial bias.

References

1. Community Service Society (2019). New Neighbors and the Over-Policing of Communities of Color. Community Service Society. https://www.cssny.org/news/entry/New-Neighbors
2. Devine, P. G., Forscher, P. S., Austin, A. J., & Cox, W. T. (2012). Long-term reduction in implicit race bias: A prejudice habit-breaking intervention. Journal of Experimental Social Psychology, 48(6), 1267–1278. doi: 10.1016/j.jesp.2012.06.003
3. Education Week Research Center (2018). School Policing: Results of a National Survey of School Resource Officers. [PDF file] Bethesda, MD. Retrieved from: https://www.edweek.org/media/school-resource-officer-survey-copyright-education-week.pdf
4. Fabelo, T., Thompson, M. D., Plotkin, M., Carmichael, D., Marchbanks, M. P., Booth, E. A. (2011). Breaking schools' rules: A statewide study of how school discipline relates to students' success and juvenile justice involvement New York, NY: Council of State Governments Justice Center.
5. Finn, P., Shively, M., McDevitt, J., Lassiter, W., & Rich, T. (2005). Comparison of Program Activities and Lessons Learned among 19 School Resource Officer (Sro) Programs. Document Number 209272.

Place of publication not identified: Distributed by ERIC Clearinghouse.
6. Ireland, D. L. (2003). The price of education: What local control is costing American children. Scholar, 6, 159.
7. Kirkland, E. (2008). What's Race Got to Do With it? Looking for the Racial Dimensions of Gentrification. Western Journal of Black Studies, 32(2).
8. Kupchik, A. (2009). Things are tough all over: Race, ethnicity, class and school discipline. Punishment and Society, 11, 291–317.
9. Laniyonu, A. (2018). Coffee shops and street stops: Policing practices in gentrifying neighborhoods. Urban Affairs Review, 54(5), 898-930. doi:10.1177/1078087416689728
10. Leguichard, S. (2019). Gentrification: Undercover Agent Of Mass Incarceration. Retrieved from: https://marcusharrisfoundation.org/blog/f/gentrification-undercover-agent-of-mass-incarceration
11. Marcuse, P. (1985). Gentrification, abandonment, and displacement: Connections, causes, and policy responses in New York City. Wash. UJ Urb. & Contemp. L., 28, 195.
12. Nicholson-Crotty, S., Birchmeier, Z., & Valentine, D. (2009). Exploring the Impact of School Discipline on Racial Disproportion in the Juvenile Justice System. Social Science Quarterly, 90(4), 1003–1018. doi: 10.1111/j.1540-6237.2009.00674.x
13. Redfield, S. E., & Nance, J. P. (2016). The American bar association joint task force on reversing the school-to-prison pipeline preliminary report. American Bar Association Coalition on Racial and Ethnic Justice, Criminal Justice Section, and Council for Racial & Ethnic Diversity in the Educational Pipeline (2016), 16-44.
14. Rocque, M., & Snellings, Q. (2018). The new disciplinology: Research, theory, and remaining puzzles on the school-to-prison pipeline. Journal of Criminal Justice, 59, 3–11. doi: 10.1016/j.jcrimjus.2017.05.002
15. Ronquillo, J., Denson, T. F., Lickel, B., Lu, Z. L., Nandy, A., & Maddox, K. B. (2007). The effects of skin tone on race-related amygdala activity: An fMRI investigation. Social cognitive and affective neuroscience, 2(1), 39-44.
16. Rose, K. (2002). Combating gentrification through equitable development. Race, Poverty & the Environment, 9(1), 5-56.
17. Rothstein, R. (2015). The racial achievement gap, segregated schools, and segregated neighborhoods: A constitutional insult. Race and social problems, 7(1), 21-30.
18. Russell J. Skiba, Robert H. Horner, Choong-Geun Chung, M. Karega Rausch, Seth L. May & Tary Tobin. (2011). Race Is Not Neutral: A National Investigation of African American and Latino Disproportionality in School Discipline, School Psychology Review, 40:1, 85-107, doi: 10.1080/02796015.2011.12087730
19. Sharp, Elaine B. (2013). "Politics, Economics, and Urban Policing: The Postindustrial City Thesis and Rival Explanations of Heightened Order Maintenance Policing." Urban Affairs Review 50 (3): 340–65.
20. Shen, F., Hu, Y., Fan, M., Wang, H., & Wang, Z. (2018). Racial Bias in Neural Response for Pain Is Modulated by Minimal Group. Frontiers in Human Neuroscience, 11. doi: 10.3389/fnhum.2017.00661
21. Sherman, L. E., Rosenbaum, G. M., Smith, A. R., Botdorf, M. A., Fettich, K., Patrianakos, J. L., Chein, J. M. (2019). The interactive effects of peers and alcohol on functional brain connectivity in young adults. NeuroImage, 197, 264–272. doi: 10.1016/j.neuroimage.2019.04.003
22. Wake, S. J., Aoki, R., Nakahara, K., & Izuma, K. (2019). Elucidating the role of the posterior medial frontal cortex in social conflict processing. Neuropsychologia, 132, 107124. doi: 10.1016/j.neuropsychologia.2019.107124
23. Welch, K., & Payne, A. A. (2010). Racial Threat and Punitive School Discipline. Social Problems, 57(1), 25–48. doi: 10.1525/sp.2010.57.1.25
24. Wharton, J. L. (2008, June). Gentrification: The new colonialism in the modern era. In Forum on Public Policy: A Journal of the Oxford Round Table. Forum on Public Policy.
25. Wheeler, M. E., & Fiske, S. T. (2005). Controlling Racial Prejudice. Psychological Science, 16(1), 56–63. doi: 10.1111/j.0956-7976.2005.00780.x
26. Vitale, A. S. (2008). City of disorder: How the quality of life campaign transformed New York politics. NYU Press.

27. Zuk, Miriam, Bierbaum, Ariel H., Chapple, Karen, Gorska, Karolina, Loukaitou-Sideris, Anastasia, Ong, Paul, Thomas, Trevor. (2015). "Gentrification, Displacement, and the Role of Public Investment: A Literature Review." Technical Report, Federal Reserve Bank of San Francisco. http://urbandisplacement.t324.com/sites/default/files/images/displacement_lit_review_final.pdf.
28. Zukin, Sharon. Naked city: The death and life of authentic urban places. Oxford University Press, 2009.

Meet Our Editing Team

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Julia Davis,
President & Editor-in-Chief

Julia Davis is a junior 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 a Family Medicine doctor. 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,
Senior Editor

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,
Senior Editor

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.

PEER REVIEW TEAM

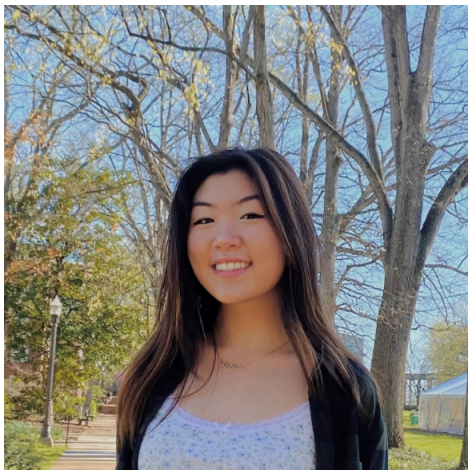
Julian Burbano

Julian is a Junior from Northern Virginia majoring in Evolutionary Anthropology and minoring in Music and Chemistry and is on the Pre-Med track. He does research in the Adams Lab studying cellular bioprinting and 3D-printed orthopedic implants. Julian has thoroughly enjoyed being a part of the Vertices Journal and looks forward to seeing the amazing work that will come through in the future!

Colby Cheshire

Colby is a junior studying French and Biology with a concentration in genetics. He has previously worked in the Alberts Lab at Duke and the Speliotes Lab at the University of Michigan. Outside of lab and class, Colby enjoys volunteering with Crisis Text Line, watching movies, and discovering new coffee shops in Durham.

Arielle Kim

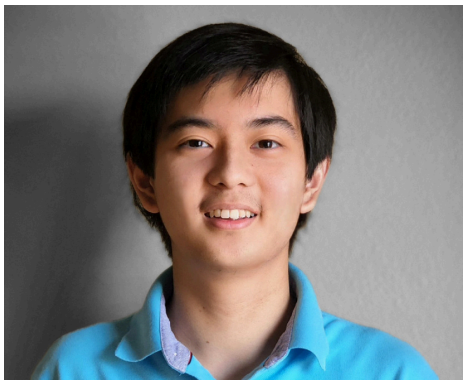


Arielle (Trinity ’26) is a prospective biology major interested in genetics and its intersection with ecology. She is involved as a peer reviewer at Vertices and is also a member of Duke STEM Connect, a club dedicated to supporting STEM curricula in local schools. She is also a huge animal lover, so she volunteers as a Technician Assistant at the Duke Lemur Center on the weekends.

Eliza Goldstein

Eliza (Trinity ’26) is a freshman from New York, planning to major in Psychology with a minor in Global Health. She is interested specifically in social and clinical psychology, working in the Duke Culture Lab and the Zucker Lab. Outside of Vertices, she enjoys biking, tutoring, and spending time with her friends.

Eric Lee



Eric Lee is a freshman from Florida planning on studying computer science. He is especially interested in the intersections of machine learning with medicine and clinical psychology. In his free time, he loves playing ping-pong and reading.

Katherine Long

Katherine (Trinity ‘24) is a junior majoring in biology and chemistry with a concentration in cell and molecular biology. She is passionate about scientific communication and research and is excited to contribute to Vertices as a staff writer and peer reviewer. When she’s not in the lab or doing homework, she loves to paint, hike, and hang out with the Duke cat.

Anya Milberg

Anya Milberg is a freshman from New York City majoring in Neuroscience with a certificate in Child Policy Research. She is very interested in exploring the intersection of science and the law, particularly as it pertains to free will, mental health, and culpability. Outside of Vertices, she can be found leading tours of campus, volunteering with the Duke Justice Project, and spending time with friends.

Aditya Raj

Adi is an undergraduate studying biology, global health, and data science. His research at Musah lab involves studying the molecular signals and biophysical forces involved in iPSC generation to guide podocyte development for therapeutic uses. In his free time, Adi plays squash, listens to jazz, and volunteers with Meals on Wheels.

Asher Wallen

Asher Wallen is a first-year Pratt student with the intention to major in biomedical engineering and minor in mathematics and French studies. He was born in Mt. Pleasant, South Carolina, and has lived there his entire life. He is also a member of Duke Brimstone (club ultimate frisbee), Duke chess, DukEngineer, Duke Diya, and Duke STEM Connect.

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.

Meet Our Design Team

AJ Kochuba,
Artistic Director

AJ (Trinity ‘25) is a sophomore 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, competing on the pickleball courts, and performing in dance showcases.

Erin Heyeck

Erin (Trinity ‘24) is a Junior 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.

Cindy Ju

Cindy is a freshman 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.

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