Biological Factors Contributing to Gender Diversity

By

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Paper submitted in partial fulfillment of the requirements for the degree of

Master of Counselling
in the
Division of Arts and Sciences

City University
of Seattle
2025

This paper is accepted as conforming to the required standard.

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Date

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Acknowledgements

I am grateful for the wonderful people who helped make this project possible. To my wife, Kim Smolley, and my daughter, Nova, thank you for your encouragement, patience, love, and for being my refuge through every challenge along the way. To my mom, Carolyn Smolley, thank you for celebrating me at every step and for always believing in me. To Yves Cloutier, whose weekly company, laughs, kindness, and loyalty gave me the space to reset and return to this work with a clearer mind, thank you. Thank you to my friends and mentors Kate Schneider, Katelyn Mayer, Laura McLaughlin, and Ingrid Pederson. Each of you has shaped the person, counsellor, and researcher I am becoming, and I carry your influence forward with gratitude. To Erika Goos, your guidance and humour carried me farther than you know and helped me reach the finish line with my sanity still intact. Finally, a heartfelt thank you to Dr. De Guerre. I am proud of this paper, and your mentorship played a major role in that, especially your insistence that there is always a deeper synthesis to find, and that writing can always stretch a little further.

Abstract

Gender diverse individuals face elevated risks of stigma, discrimination, and mental health concerns, while counselling psychology continues to lack a unified, biologically informed framework to guide affirming practice. This capstone addresses the research question: Which biological factors contribute to gender diversity, and how can synthesis of this knowledge support affirming, evidence-based counselling practice?

This capstone employs an integrative literature review to synthesize biological and neurodevelopmental research relevant to gender diversity, drawing on 27 peer-reviewed articles published between 2005 and 2024. Through inductive synthesis guided by neurodevelopmental theory, recurring molecular, cellular, and developmental mechanisms became apparent, allowing the review to trace how interacting biological systems shape gendered outcomes during prenatal development.

Findings demonstrate that brain and body follow partially distinct developmental timelines and that multiple, interacting molecular systems support a spectrum of gendered outcomes rather than a fixed binary. This paper will translate this synthesis into counselling applications by linking biological knowledge with case conceptualization, psychoeducation, advocacy, and supervision, and by proposing the Neurodevelopmental Approach to Gender Affirmation (NAGA) as an applied extension of existing gender affirming models.

NAGA organizes practice around three core tasks: exploration, validation, and integration of biological narratives in ways that remain client-led, culturally responsive, and ethically grounded. The capstone concludes with recommendations for future research, and highlights implications for training, ethical practice, and policy. Overall, this literature analysis paper

positions gender diversity as a natural expression of human neurodevelopment and offers counsellors biologically informed tools to support affirming, evidence-based care.

Keywords: gender diversity; neurodevelopment; gendered neurological differentiation; prenatal development; biological factors; counselling psychology; gender-affirming care; neurodevelopmental theory; biological psychoeducation; microglia.

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Chapter 1: Introduction

Background

Gender diversity refers to all genders, including those that exist outside of the cisgender binary (Coleman et al., 2022). Yet despite growing awareness, counselling psychology continues to lack a unified synthesis of biological research that explains gender diversity in ways that inform practice. This gap limits practitioners' ability to provide empirically grounded, affirming care. The core clinical issue addressed in this capstone is counselling psychology's lack of biologically informed, affirming models to guide competent care for gender diverse clients.

Expressions that diverge from cultural norms often expose individuals to stigma and discrimination (Bowers & Whitley, 2020). In this paper, stigma refers to the prejudices that provide the foundation for social, interpersonal, societal, and institutional processes that delegitimize gender diversity, including discrimination, harmful narratives, exclusion, misinformation, harm, and pathologization that undermine mental health and help-seeking (MacKinnon et al., 2025). These processes elevate risks of anxiety, depression, substance use, and suicidality (Bowers & Whitley, 2020; Carone et al., 2021; Ferlatte et al., 2021; Johns et al., 2019; Nguyen et al., 2019).

These harms are compounded by institutional gatekeeping practices such as transnormative models that classify gender diversity as pathology and prioritize binary transitions that delay or deny access to affirming pathways of care (MacKinnon et al., 2025; Spencer et al., 2021). Together, stigma and systemic barriers create a reinforcing cycle that undermines mental health and erodes trust in helping systems (MacKinnon et al., 2025). Systemic barriers refer to structural obstacles including restrictive practices, institutional

inequities, and barriers to affirming care that limit access to competent supportive services for gender diverse individuals (Spencer et al., 2021).

Practitioners are uniquely positioned to disrupt this cycle, yet practitioner under preparedness limits their ability to integrate current biological and developmental research (Valentine & Shipherd, 2018). Without this grounding, counselling psychology risks reinforcing outdated, pathologizing frameworks rather than advancing affirming practice (Bowers & Whitley, 2020; MacKinnon et al., 2025). Synthesizing biological evidence helps break this cycle by equipping practitioners to integrate empirical knowledge into case conceptualization, psychoeducation, and advocacy, thereby supporting identity exploration and fostering family and community acceptance (MacKinnon et al., 2025; Pullen Sansfaçon et al., 2021). Specific to Canada, this literature analysis contributes directly to counselling psychology by supporting practitioner training, aligning with Canadian Psychological Association standards of practice (CPA) ethical standards, and informing policy and practice consistent with the College of Alberta Psychologists (CAP) guidelines for sufficient professional knowledge (CAP, 2023; Canadian Psychological Association [CPA], 2017).

Research Problem Statement

Counselling psychology continues to struggle with how best to address the institutional and societal challenges faced by gender diverse individuals (MacKinnon et al., 2025; Spencer et al., 2021). Practitioner under preparedness, as used in this paper, refers to limited competence, lack of training in biological and developmental research, insufficient grounding in gender affirming models, and reliance on outdated or pathologizing frameworks. This under preparedness manifests in reliance on transnormative models and gatekeeping practices that

misclassify gender diversity as pathology and fail to support nonbinary clients (Bowers & Whitley, 2020; MacKinnon et al., 2025; Spencer et al., 2021).

Existing reviews tend to focus narrowly on discrete mechanisms such as hormones, genetics, or neuroanatomy, often implying possible connections between them without fully integrating these findings. Others emphasize sociocultural perspectives but overlook biological pathways, leaving the evidence fragmented. As a result, the field lacks a comprehensive, clinically relevant synthesis that counsellors can readily apply in practice. Without such integration, practitioner under preparedness persists, limiting counsellors' ability to counter misinformation, advocate for individualized care, or confidently incorporate biological insights into therapeutic work (Abreu et al., 2022).

This absence of integration has direct consequences for professional practice. Counsellors require accessible, evidence-based frameworks that link biological and developmental science to affirming, client-centred care. A synthesized understanding of the biological foundations of gender diversity strengthens practitioner competence, enhances psychoeducation with clients and families, and supports advocacy within systems that continue to marginalize gender diverse individuals.

Delimitations

For the purposes of this capstone, biological factors refer to any molecular, cellular, or developmental contributors that interact with human biology and influence gendered neural differentiation (McCarthy et al., 2018, VanRyzin et al., 2020). The synthesis is intentionally limited to prenatal and early developmental mechanisms and does not incorporate adult hormone therapy outcomes, genomic sequencing datasets, neuroimaging evidence, behavioural or experiential data, or psychosocial or sociocultural explanations of gender diversity. Studies were

included only when they contributed directly to a molecular or cellular account of developmental processes. This literature analysis does not aim to provide a comprehensive review of all biological research but focuses on select biological factors that offer the strongest relevance for counselling psychology.

I will address both needs by synthesizing current biological and neurodevelopmental research through a lens tailored to counselling psychology. This lens establishes a sequential account of key molecular processes that contribute to gender diversity while demonstrating how these findings connect to practical considerations in clinical work. Unlike earlier reviews that often focus on isolated mechanisms, I will integrate evidence across molecular, hormonal, and developmental levels, and present the material in both scientific and accessible language so counsellors can translate complex findings into practice with greater confidence.

This literature analysis addresses two related research questions: “Which biological factors contribute to gender diversity?” and “How can a synthesized understanding of these biological factors inform affirming, evidence-based, and client-aligned counselling practice for gender diverse individuals?”

The first question is addressed directly through the integrative review and synthesis of biological and neurodevelopmental research presented in Chapter 3. The second question is addressed through the structure of the capstone as a whole, rather than through the literature review alone. Specifically, Chapter 4 translates the biological synthesis into clinical applications, demonstrating how these findings can be integrated into case conceptualization, psychoeducation, advocacy, and ethical practice within counselling psychology.

Rationale

Access to competent, affirming care remains limited for gender diverse individuals, with long wait times, a shortage of knowledgeable practitioners, and pressures toward binary transition pathways (Javier et al., 2024). These systemic barriers contribute to delayed or inappropriate care, often leading to preventable mental health crises (Bowers & Whitley, 2020; Javier et al., 2024).

This paper aims to address these gaps by presenting biological evidence that supports gender as a spectrum and demonstrating the need for additional individualized gender-affirming care pathways (Javier et al., 2024). By compiling this research, the study seeks to reduce practitioner under preparedness, improve competency, and prevent avoidable harms to clients. This aligns with counselling psychology's role in social justice advocacy by equipping practitioners with empirically grounded tools to challenge stigma, resist systemic inequities, and support marginalized communities (Canadian Psychological Association [CPA], 2017). While this rationale highlights the pressing need to close existing knowledge gaps, the significance extends further, influencing how the profession adapts to emerging demographic realities and how counsellors translate affirming principles into everyday practice.

Significance

The number of gender diverse individuals is increasing with each new generation (Carone et al., 2021; Johns et al., 2019). Current U.S. estimates suggest the overall gender diverse population makes up 0.6% of the total, compared to 2% of all adolescents who are gender diverse (Carone et al., 2021; Johns et al., 2019). This demographic shift makes it increasingly likely that counsellors will encounter gender diverse clients many of whom are at elevated risk of mental health concerns due to discrimination, abuse, and stigma (Carone et al., 2021; Johns et al., 2019; Valentine & Shipherd, 2018).

Knowledge of the biological basis of gender diversity can improve acceptance by attributing diversity to stable internal factors, which correlate with greater family and community support (Bowers & Whitley, 2020). For counsellors, this knowledge could have concrete applications in daily practice. It informs case conceptualization by allowing practitioners to integrate biological evidence into treatment planning, enhances psychoeducation by equipping them to explain complex findings in accessible ways to clients and families, and strengthens advocacy efforts by providing empirically grounded arguments when working with schools, medical providers, or policy systems (Bowers & Whitley, 2020; Pullen Sansfaçon et al., 2022; Spencer et al., 2021).

The gender affirmative lifespan approach (GALA) provides a therapeutic model for counselling gender diverse clients and stands to benefit from biological knowledge that clarifies part of how gender diversity emerges (Spencer et al., 2021). Its core tenets, affirming care, gender literacy, empiricism, intersectionality, and moving beyond the binary, are reinforced when practitioners can situate the clients' experiences within established biological pathways (Spencer et al., 2021). This evidence helps recognize gender diversity as part of the natural human spectrum, enables clients to critically evaluate and externalize harmful narratives from medical and social systems, and reinforces intersectionality by underscoring the variability of gendered experience (MacKinnon et al., 2025; Spencer et al., 2021). It also equips counsellors with empirically grounded language for case conceptualizations, treatment planning, and collaboration with medical professionals (Spencer et al., 2021).

Without this integration, counselling psychology risks perpetuating outdated, binary-focused models of care that undermine client well-being (MacKinnon et al., 2025). A detailed molecular account of how gender diversity arises leaves less room for debate about its

legitimacy, can strengthen counsellor confidence when advocating for clients, and supports efforts to expand affirming care. By embedding this synthesized biological evidence into counsellor education, training programs can prepare practitioners to combat stigma, support identity exploration, and provide high-quality quality affirming care (Abreu et al., 2022; Bowers & Whitley, 2020; Javier et al., 2024; MacKinnon et al., 2025; Pullen Sansfaçon et al., 2022; Spencer et al., 2021).

This synthesis also has implications for curriculum design and clinical training in Canadian counselling psychology, as it can inform supervision practices, practicum experiences, and graduate education by embedding biological perspectives into the preparation of future practitioners (Spencer et al., 2021). Beyond practice and training, the findings of this synthesis can inform future research agendas by identifying underexplored biological mechanisms and by shaping policy discussions around standards of affirming care, evidence-based training, and equitable access for gender diverse populations. Taken together these points establish why the present study matters, but understanding how its insights can be meaningfully applied in counselling practice requires grounding in a theoretical framework that situates biological processes in a developmental context and enables their translation into counselling practice.

Theoretical Frameworks Guiding This Research

Neurodevelopmental Theory

This literature analysis applies neurodevelopmental theory as a guiding theoretical framework to examine how biological processes contribute to gender diversity. Neurodevelopmental theory emerged within developmental neuroscience and psychiatry in the late twentieth century to explain how genetic, hormonal, and environmental factors interact during sensitive and critical periods of brain development to shape long-term outcomes,

including vulnerability to psychiatric conditions (Gałecki & Talarowska, 2018; Dehorter & Del Pino, 2020). Although neurodevelopmental theory was not developed specifically to explain gender, its modern formulation is often traced to psychiatric research demonstrating early developmental origins of later outcomes (Murray et al., 2017). Weinberger's (1987) model of schizophrenia as a disorder of early brain development with delayed clinical expression is frequently cited as a key consolidation of this framework (Murray et al., 2017).

Over time, neurodevelopmental theory has been extended to research on sexual differentiation of the brain, where it is used to account for how timing-dependent molecular and cellular processes influence the organization of gendered neural circuits (McCarthy et al., 2018; VanRyzin et al., 2020; Bakker, 2022). Within this literature, neurodevelopmental theory postulates that variations in genetic signaling, hormone exposure, receptor sensitivity, epigenetic regulation, and cellular processes during critical prenatal windows can produce enduring differences in neural organization (Dehorter & Del Pino, 2020; Doi et al., 2022; Gałecki & Talarowska, 2018).

In this project, neurodevelopmental theory provides the structural framework for organizing and synthesizing biological research across genetics, hormones, receptor polymorphisms, epigenetics, microglia, and environmental influences. It guides the sequencing of the literature in Chapter 3 into a developmental account that explains how variation in early neurodevelopmental processes can contribute to a spectrum of gendered outcomes. This framework is therefore used to address the first research question by clarifying which biological factors contribute to gender diversity and how they interact during critical developmental periods. This theory was chosen over biopsychosocial or sociocultural models, such as queer theory, because it provides a direct molecular and developmental account of how gendered

neurological differentiation emerges during prenatal life, while still allowing later integration with social and psychological perspectives (Dehorter & Del Pino, 2020).

Because neurodevelopmental theory explains biological mechanisms but does not address how such knowledge should be interpreted or applied within clinical contexts shaped by stigma, marginalization, and ethical responsibility, a complementary framework is required.

Transformative Framework

This literature analysis is also guided by a transformative framework that addresses the ethical and social conditions under which knowledge is produced and used, particularly in contexts shaped by power, marginalization, and historical misuse (Creswell & Creswell, 2022; Creswell & Poth, 2016). Within counselling psychology, this framework emphasizes reflexivity, social justice, and the responsible translation of research into practice, especially when working with historically marginalized populations such as gender-diverse individuals.

In relation to the research problem identified in this study, the transformative framework serves to mitigate the risk that biological explanations of gender diversity may be misinterpreted or misused to reinforce pathologization, gatekeeping, or binary norms (Creswell & Creswell, 2022; Creswell & Poth, 2016; Spencer et al., 2021). While neurodevelopmental theory provides an explanatory account of how biological processes contribute to gender diversity, the transformative framework guides how the biological synthesis developed in Chapter 3 is translated into counselling practice, ensuring that biological findings are framed and applied in ways that support affirming, evidence-based, and client-aligned care (Canadian Institutes of Health Research, Natural Sciences and Engineering Research Council of Canada, & Social Sciences and Humanities Research Council, 2022; Creswell & Creswell, 2022).

Definition of Key Terms

The Gender Spectrum

The gender spectrum posits that gender cannot be constrained to two binary categories (Spencer et al., 2021). Instead, gender lies on a continuum shaped by emotional, biological, behavioral, and cognitive characteristics (Coleman et al., 2022).

Gender Diverse Individuals

Gender diverse individuals are those whose identities differ from their sex assigned at birth including transgender, nonbinary, and identities beyond these categories (Coleman et al., 2022). This project uses gender diversity as an inclusive term to reflect the broad range of identities under study and to align with its transformative framework (Coleman et al., 2022).

Gender Identity

Gender identity refers to one's internal, deeply held sense of being located anywhere within or outside the gender spectrum and remains distinct from gender expression and gender roles (Spencer et al., 2021).

Gender Literacy

Gender literacy refers to the ability to critically recognize, interpret, and evaluate societal gender narratives grounded in an understanding of the social, experiential, and biological factors that shape an individual's sense of gender (Spencer et al., 2021).

Affirming Care

Affirming care refers to clinical practice that recognizes, respects, and supports the gender identities that clients state or express, providing treatment that aligns with and validates those identities (Mendoza et al., 2020).

Gendered Neurological Differentiation (GND)

GND refers to the cellular and molecular processes by which the brain differentiates based on hormonal, epigenetic, and genetic factors during development which occur during critical prenatal windows to influence gendered neural trajectories (Bakker, 2022; McCarthy et al., 2018; Nelson et al., 2019; VanRyzin et al., 2020).

Differences in Sexual Development

DSDs involve genetic, epigenetic, hormonal, or anatomical variations in sexual development. Here, they provide a natural context for examining how atypical developmental pathways inform our understanding of gendered neurodevelopment (Elzaiat et al., 2022; Okashita & Tachibana, 2021).

Critical Periods

Critical periods are windows of heightened brain plasticity when environmental, and biological signals have amplified influence on development (McCarthy et al., 2018; Dehorter & Del Pino, 2020). This study highlights critical periods as the developmental windows where biological diversity in gender emerges (McCarthy et al., 2018).

Microglia

Microglia are glial cells that shape neural development and are responsive to a range of neuroendocrine and molecular signals, including hormones, neurotransmitters, endocannabinoids, and endocrine disruptors (VanRyzin et al., 2020; Bobotis et al., 2023). In this project, they exemplify how cellular processes within the brain contribute to gendered neurological differentiation.

Endocrine Disruptors

Endocrine disruptors are environmental agents that can interfere with hormone activity, and epigenetic regulation during fetal development (Gore et al., 2014; Santoro et al., 2021;

Solleiro-Villavicencio et al., 2020). They are discussed here to illustrate how environmental influences intersect with biological pathways in shaping gender diversity.

Researcher Reflexivity and Positionality

As a gender diverse individual, I have personally experienced barriers to competent gender affirming care, including difficulty finding affirming, knowledgeable therapists and navigating binary gatekeeping in medical systems. These experiences, along with exposure to stigma and misinformation in psychological, medical, and political contexts help shape my commitment to advancing empirical knowledge on the biological basis of gender diversity.

My work during my internship with gender diverse clients has shown me how integrating biological research into practice can affirm identities, challenge misconceptions, and support individualized care. I recognize that both my lived experience and professional commitment may introduce bias into how I select, interpret, and present the literature. To mitigate this, I will maintain a reflexive journal, seek supervisory feedback, and remain open to alternative interpretations.

At the same time, I anticipate potential tensions in this work. For example, a scientific framing that emphasizes neurodevelopmental and molecular mechanisms may be perceived as reductive when compared with lived experience, which is complex, fluid, and situated in sociocultural contexts (Valentine & Shipherd, 2018). Similarly, choosing the neurodevelopmental framework over sociocultural or critical models could create friction, as it privileges biological explanations that may not fully capture the roles of identity, community, and systemic factors (Spencer et al., 2021). By naming these tensions explicitly, I aim to approach the integration of biological evidence with care, recognizing that no single framework can exhaustively account for gender diversity (Creswell & Creswell, 2022; Spencer et al., 2021).

In qualitative and mixed methods research, researcher positionality is recognized as a critical factor in shaping both the process and outcomes of inquiry (Creswell & Creswell, 2022). Acknowledging my lived experiences and values ensures that readers can evaluate how these factors may have influenced my interpretations. This transparency aligns with Tri-Council Policy Statement 2 (TCPS 2) and CPA ethical standards including Principle I standards I.7 and I.10, and Principle II standards II.5, II.10, II.17 and II.32, by emphasizing respect for persons and responsible caring (Canadian Institutes of Health Research, Natural Sciences and Engineering Research Council of Canada, & Social Sciences and Humanities Research Council of Canada, 2022; CPA, 2017). In this way, the reflexivity process reflects a commitment to preserve respect for the dignity of gender diverse communities, minimize the risk of misrepresentation or harm in how findings are communicated, and promote fairness and justice in the production and application of knowledge (CPA, 2017).

Reflexivity will also be revisited throughout the study, particularly in Chapter 5's reflections, to evaluate how my perspectives and interpretations evolved across the research process, ensuring ongoing transparency and self-awareness in interpreting and applying the findings.

Summary

Gender diverse individuals continue to face stigma and systemic barriers that limit access to competent, affirming care (Carone et al., 2021; Johns et al., 2019; Valentine & Shipherd, 2018). These difficulties are compounded by misinformation in medical, psychological, and political systems, which contributes to poor mental health outcomes (Abreu et al., 2022; Bowers & Whitley, 2020). This research seeks to provide practitioners with an integrated, evidence-based understanding of the biological factors underlying gender diversity, equipping them to counter

misinformation, advocate for individualized care, and foster acceptance (MacKinnon et al., 2025; Pullen Sansfaçon et al., 2022; Spencer et al., 2021).

Guided by neurodevelopmental theory, this literature analysis aims to demonstrate how biological processes interact during critical periods of development to produce the diversity of gender identities observed across the spectrum (Dehorter & Del Pino, 2020; Doi et al., 2022; Gałecki & Talarowska, 2018; Santos et al., 2022). This knowledge equips counsellors to apply biological evidence in case conceptualization, advocacy, and psychoeducation, ensuring that affirming practice is both scientifically grounded and client centered.

To strengthen the overall coherence of the capstone, the objectives introduced in Chapter 1 guide the structure of the chapters that follow. Chapter 2 builds on the problems identified here, specifically practitioner under-preparedness and the need for a unified biological synthesis, by outlining the integrative review design and concept-driven search strategy used to address them. This integrative review design is then used in Chapter 3 to synthesize the selected literature into a sequential, neurodevelopmental account of how genetics, hormones, epigenetics, microglia, and environmental factors interact during critical periods of development.

Chapter 4 responds to the practical need for accessible, evidence informed frameworks by translating the biological synthesis into counselling applications aligned with ethical standards and current models of gender affirming care. Chapter 5 concludes the project by revisiting the aims introduced in Chapter 1, summarizing the study's contributions, discussing implications for practitioner competence and training, acknowledging methodological limitations, and identifying future directions for advancing affirming, biologically informed counselling practice.

Chapter 2: Methods of Literature Research

Literature Search Process

This chapter outlines the integrative literature review methodology used to identify, evaluate, and synthesize biological and neurodevelopmental research relevant to gender diversity. The intent is to orient readers who may be less familiar with integrative review designs by clarifying how the search strategy, inclusion and exclusion criteria, and critical synthesis procedures were structured to inform counselling psychology practice.

An integrative literature **review** methodology was employed to guide this research. This approach was chosen because it allows the summarization of the body of research to date on the biological aspects of gender diversity, the synthesis of findings across disciplines, the identification of potential avenues for new research, and the critique of limitations in the current body of knowledge (Torraco, 2016). The scope of this review is representative rather than exhaustive. For example, while there is an extensive body of literature on hippocampal differentiation, reviewing and summarizing all such studies would exceed the scope of this capstone. Instead, the most relevant and influential studies were selected to illustrate key mechanisms and interactions (Torraco, 2016). Similarly, important insights into neurological differentiation related to gender often emerge from research not explicitly focused on gender diverse individuals, such as broader developmental neuroscience, which further expands the potential body of sources (Mu et al., 2020).

An integrative review design was selected because it emphasizes critical synthesis and the generation of new conceptual perspectives across diverse forms of evidence (Torraco, 2016). In contrast, scoping reviews focus on mapping the range and characteristics of existing research rather than critically appraising or synthesizing findings into a unified conclusion (Munn et al.,

2018). A systematic review was also not feasible or conceptually appropriate given the extensive breadth and heterogeneity of evidence on gendered neurological differentiation. As Shaheen et al. (2023) note, systematic reviews and meta-analyses require a certain degree of methodological and topical homogeneity and combining studies with widely differing designs or outcomes can lead to misleading or meaningless results. The integrative approach provided the necessary flexibility to connect findings from molecular, endocrinological, and neurodevelopmental research into a coherent explanatory framework for understanding how biological processes contribute to gender diversity.

The database search strategy followed an iterative, concept-driven approach that balanced conceptual breadth and precision through mixed Boolean structures, ensuring balanced retrieval of literature spanning gender, neurodevelopmental, and biological domains (Torraco, 2016). Search terms were developed and organized into six conceptual domains to guide searches in PsycINFO, PubMed, and ScienceDirect, aiming to identify literature on biological and neurodevelopmental processes relevant to gender diversity. These domains included: A. Gender Diversity ("gender diversity," "gender spectrum," "gender identity," transgender, "nonbinary," or "differences in sexual development"); B. Neurodevelopmental Processes (neurodevelopment, "critical periods," "neurological differentiation," "sexual differentiation," "neurological sex differences," or "sex differences"); C. Biological Mechanisms (genetics, epigenetics, receptors, hormones, estrogen, androgen, or endocannabinoids); D. Prenatal Development (fetus, "fetal development," or prenatal); E. Environmental Factors ("endocrine disruptors" or "environmental teratogens"); and F. Glial and Neural Cells ("glial cells," astrocytes, or microglia).

Boolean configurations were refined through iterative cycles of searching, reviewing, and adjusting to explore how different combinations of concepts influenced scope and relevance

(Torraco, 2016). The primary configuration was A AND (B OR C OR D OR E OR F), with rotations (D AND B AND (A OR C OR E OR F); D AND (A OR B OR C OR E OR F); B AND (A OR C OR D OR E OR F)) used to assess how shifting the anchor term altered the types of studies retrieved. In PubMed and PsycINFO, search terms were limited to the title and abstract fields to ensure that retrieved articles focused on these concepts rather than mentioning them incidentally. These parameters reduced excessive counts of results and enabled more targeted screening of relevant studies.

Because ScienceDirect operates with stricter Boolean and character limitations (maximum of eight connectors and a lower number of characters per query), search strings were condensed into broader groupings of gender, neurodevelopmental, hormonal, and glial terms. These simplified configurations represented each biological domain within a single search structure, maintaining conceptual coverage while staying within database limits. However, reducing the number of terms within each group also limited the ability to capture studies that used similar but distinct terminology, slightly decreasing the likelihood of discovering all relevant articles. Terms could not be confined to specific parts of each article, such as only the title or abstract, resulting in broader searches that prioritized coverage over precision. This wider reach increased the number of results but also captured papers where key terms appeared only briefly.

Within ScienceDirect, a combination of AND and OR operators was used to balance conceptual breadth and precision. Nested ORs captured variation within each biological domain, while ANDs preserved intersections between major concepts such as gender, neurodevelopment, and prenatal processes. This structure maintained the conceptual scope of the broader search

strategy while reducing combinatorial depth, ensuring that results reflected the integrative framework despite the database's Boolean and character limits.

The overall search process was iterative and concept-driven, aligning with recommendations for integrative literature reviews, which emphasize theoretical synthesis developed through cyclical refinement (Torraco, 2016). Search terms were initially broad and repeatedly tested in combination, then expanded and reorganized as emerging patterns revealed which conceptual links produced the most relevant results. The Boolean configurations presented in Appendix A represent the final iteration of this process. Following completion of the database searches, citation chaining and snowballing were applied to identify additional relevant works through reference lists and forward citations, further strengthening coverage of key biological and developmental themes.

Table 1

Database Search Strategy Overview

Database	Filters	Boolean Group Combinations	# of Results for all search terms	#Included
PubMed	Meta Analysis, Review, Scoping Review, Systematic Review, English, 2005–Present, Abstract, Title	A AND (B OR C OR D OR E OR F); rotations: D AND B AND (A OR C OR E OR F); D AND (A OR B OR C OR E OR F); B AND (A OR C OR D OR E OR F)	8198	14
PsycINFO	After January 2005, Journal, Journal Article, Review Book, English, Peer Reviewed, Abstract, Title	A AND (B OR C OR D OR E OR F); rotations: D AND B AND (A OR C OR E OR F); D AND (A OR B OR C OR	8535	4

		E OR F); B AND (A OR C OR D OR E OR F)		
ScienceDirect	2005–2024, Review Articles, Research Articles, English, Neuroscience, Environmental Science, Biochemistry Genetics and Molecular Biology, Pharmacology Toxicology and Pharmaceutical Science, Immunology and Microbiology	A AND (B OR C OR F); rotations: D AND (B OR A OR C); D AND (C OR F); B AND (A OR F); B AND C AND D; E AND D AND (A OR B OR C OR F)	12924	6
Snowballing and Chaining	N/A	Reference Lists of Found Articles	N/A	3

Inclusion Criteria

The inclusion and screening process for this review occurred in two stages. Search results from each database were first screened by title and abstract for relevance to the inclusion criteria, with duplicates and clearly unrelated papers not included. Because the combined initial yield across all databases was approximately 29,657 results, only the top-ranked and most relevant results within each database were examined in depth. The number of records screened varied, as some queries produced multiple pages of relevant studies while others reached saturation after only a few pages. In the second stage, sources were re-evaluated by publication date to prioritize the most recent studies and replace older works with newer research where possible, while retaining foundational studies when no updated equivalents existed. This process resulted in 27

peer-reviewed papers that met all criteria, forming the final body of literature integrated into the review.

Peer-reviewed articles, reviews, and meta-analyses that directly addressed biological, neurological, or developmental contributions to gender diversity were prioritized. The timeframe of 2005–2024 was selected to capture contemporary findings while allowing inclusion of seminal works that have not yet been expanded upon. This period encompasses the era in which key neurodevelopmental and endocrinological research clarified the timing of brain and body differentiation, providing essential context for interpreting more recent discoveries. The review therefore adopted a balanced temporal approach, integrating current data with foundational studies that continue to inform existing models.

Whenever possible, research that addressed the full spectrum of gender diversity was given top priority, followed by studies that included binary transgender participants, and lastly, cisgender-only samples, which were considered when their findings could illuminate underlying molecular mechanisms that would also apply to gender-diverse individuals. Studies involving human participants were also prioritized though animal studies were included when they provided essential molecular or developmental insights not ethically obtainable in humans.

These criteria reflect an intention by the author to highlight biological mechanisms as one component of the broader story of gender diversity, grounded in the author's perspective as a counselling researcher integrating neuroscience with applied practice. This stance maintains awareness of the limitations of reductionist approaches and emphasizes the importance of interpreting biological findings within developmental, environmental, and psychosocial contexts. It acknowledges that while neurobiological research offers essential insight into the mechanisms

shaping gendered differentiation, such evidence gains meaning only when considered alongside the lived experiences, identities, and social realities of individuals.

Although the review draws conceptually from multiple disciplines, studies were included only when they contributed directly to a molecular or cellular step-by-step account of the biological processes through which the brain and body differentiate along gendered pathways. Mixed methods or psychosocially oriented studies were excluded, not due to lack of relevance, but because the scope of this project was already extensive when focused solely on biological mechanisms. Additionally, no biomolecular studies were identified that empirically incorporated social or psychological variables within the same design. This boundary-maintained depth and coherence within the biological focus of the review while recognizing a trade-off between precision and interpretive breadth, an inherent limitation when privileging depth in interdisciplinary areas such as gendered neurodevelopment.

Exclusion Criteria

The exclusion criteria excluded nonscholarly sources such as blogs, opinion pieces, and popular media, as well as theses and dissertations, which, while often rigorous, are not typically peer-reviewed. Studies that focused exclusively on social or cultural explanations of gender without reference to biology were excluded as the aim of this capstone was to examine biological contributors. Papers limited to adult hormone therapy outcomes were excluded as the emphasis of this project is on prenatal and developmental biological processes rather than medical interventions in adulthood.

Following application of the inclusion and exclusion criteria, records identified from database searches were reviewed for relevance to biological and neurodevelopmental mechanisms contributing to gender diversity. Priority was given to studies that advanced a clear

molecular, cellular, or developmental account of gendered differentiation. As the review progressed, emerging patterns informed continued refinement of focus, and additional sources were identified through citation chaining where appropriate. Full-text articles were assessed for conceptual contribution to the synthesis, resulting in 104 full-text articles reviewed and 27 studies meeting all criteria for inclusion. Because the search process was exploratory and concept-driven, the total number of abstracts reviewed across all searches was not enumerated, and the final synthesis reflects conceptual relevance rather than exhaustive coverage (Page et al., 2021; Torraco, 2016). A PRISMA-informed flow diagram summarizing the study identification and selection process is provided in Appendix B.

Within the final body of included literature, five conceptually central studies were identified as particularly influential in shaping the structure and interpretation of this capstone. These studies were selected because they either synthesized large bodies of neurodevelopmental evidence, introduced foundational mechanistic models, or clarified timing-dependent biological processes that informed the integration of findings across genetics, hormones, epigenetics, microglia, and environmental factors. The following section highlights these conceptually central studies and explains how each informed the theoretical framing, thematic organization, and developmental sequencing of the biological synthesis presented in Chapter 3.

Evaluation of Significant Studies

Several studies shaped the trajectory of this capstone. McCarthy et al. (2018) provided a detailed framework for understanding GND as a dynamic process characterized by both critical and sensitive periods. This review underscores the importance of timing in hormonal influences on the brain and highlights how epigenetic regulation plays a central role in closing developmental windows, thereby stabilizing gender specific outcomes. While McCarthy et al.

emphasize the role of timing and critical periods, VanRyzin et al. (2020) highlights microglial mediation of neural differentiation, suggesting that cellular mechanisms contribute to biological variability in ways that challenge reductionist hormone centered-models.

VanRyzin et al. (2020) reviewed how microglia act as both targets and drivers of GND in the brain, with a particular focus on extrinsic influences such as gonadal hormones and local microenvironmental cues. Their synthesis emphasizes that microglial activity through processes such as phagocytosis and synaptic pruning, serves as a key mediator of long term structural and behavioral gendered differences. Taken together, the frameworks proposed by McCarthy and VanRyzin illustrate the layered nature of gendered differentiation, where hormonal timing sets conditions for, but does not fully determine, the microglial sculpting of neural circuits.

Fernández et al. (2018, 2020) advanced understanding of how receptor polymorphisms demonstrate how subtle genetic variations contribute to diverse gender outcomes. In contrast to the more developmental focus of McCarthy and VanRyzin, Fernández and colleagues highlight a genomic dimension, showing that receptor level differences can modulate the sensitivity of neural tissue to hormonal or environmental inputs. More broadly, these studies illustrate that gendered neurodevelopment is not a fixed or uniform process but one that can be altered through shifts in receptor activity and epigenetic modulation. This convergence of neuroendocrine, immune, and genetic perspectives suggests that gender diversity arises through interacting molecular pathways rather than through any single causal axis.

Okashita and Tachibana (2021) contributed a complementary perspective by detailing how transcriptional and epigenetic regulation of the SRY gene determines testis development and, by extension, the broader cascade of GND. Although this work primarily addresses gonadal differentiation rather than direct neurodevelopment, it provides critical upstream insight into how

early genetic regulation sets the stage for later neural differentiation processes, linking sex determination to subsequent neurobiological divergence. When considered alongside McCarthy, VanRyzin, and Fernández, Okashita and Tachibana's findings clarify how early genomic regulation establishes developmental preconditions that interact with later hormonal, cellular, and epigenetic influences to produce varied gender outcomes. Delays or disruptions in SRY activation can lead to disorders of sex development, underscoring the central role of gene regulation in initiating gender specific trajectories. While these studies collectively provided critical insights, assembling and interpreting this literature was not without challenges, as described next.

Challenges Encountered

At times it was a necessity to rely on integrative reviews that combined the findings of dozens of studies. Without these review articles the scope of this project would have been restricted to narrower, isolated findings, and, within the constraints of this paper, it would not have been possible to trace the molecular pathway from genetic and hormonal signals through to neurodevelopmental outcomes. However, reliance on integrative reviews introduced a secondary layer of interpretation, as conclusions were shaped by prior authors' synthesis methods. This dependence can amplify existing selection biases and obscure methodological differences between primary studies, thereby limiting the precision of cross-study comparisons. Building from these synthesized works nonetheless provided a necessary foundation, allowing for a more complete picture of how biological processes contribute to the spectrum of gender diversity.

A key challenge was the disciplinary fragmentation of the literature. Research on gendered neurodevelopment is often scattered across disciplines such as neuroscience, endocrinology, developmental biology, and psychology, requiring extensive cross-referencing

and interpretive integration. This fragmentation complicates cumulative knowledge building because divergent methodologies and conceptual vocabularies hinder direct comparability. As a result, attempts at synthesis across molecular, developmental, and neuroendocrine domains become interpretively fragile, reducing the reliability of higher-order conclusions.

Terminology inconsistency posed challenges. For example, terms such as sex differences, sex determination, sexual differentiation, gendered differentiation, and gender differences are frequently conflated. This conflation complicates search accuracy, introducing selection bias and limiting the reliability of synthesis. Such inconsistency undermines construct validity by obscuring whether reported effects are biological, sociocultural, or interactional in nature.

Another challenge was the binary framing prevalent in many studies. Much of the literature compares cisgender male and female groups, excluding or marginalizing nonbinary and transgender participants. This limited the ability to directly assess gender diversity in many papers, though insights from these studies remain valuable for understanding underlying molecular mechanisms. Binary framing also functions as a structural bias that narrows analytic categories to cis male–female comparisons. This constraint limits representativeness and interpretive validity, reducing the applicability of findings to gender-diverse populations and perpetuating methodological binarism in biological research.

Finally, access to the most recent research on environmental factors (e.g., endocrine disruptors, cannabis exposure) was complicated by the ongoing politicization of gender studies. The politicization of gender studies introduces a risk of distorting empirical inquiry through value-laden narratives (Abreu et al., 2022; MacKinnon et al., 2025). Maintaining interpretive neutrality therefore requires heightened methodological scrutiny to separate empirical evidence from ideological framing. This process is essential to preserve both scientific integrity and

ethical responsibility, ensuring that interpretations remain evidence based and aligned with the principle of responsible caring (CPA, 2017). Given today's climate it is crucial to reject narratives that portray gender diversity as solely the result of environmental contaminants, claims that risk implying preventability or pathology, and to uphold ethical principle two of responsible caring by preventing the misinterpretation or misuse of scientific findings (Abreu et al., 2022; CPA, 2017; Bowers & Whitley, 2020; Solleiro-Villavicencio et al., 2020; VanRyzin et al., 2020).

Methodological Limitations

The methodology of this literature search carries several limitations. First, while preference was given to the most recent studies, some areas of inquiry (e.g., prenatal androgen bursts) rely on older but still foundational research due to limited updated findings (Bao & Swaab, 2011; Swaab & Garcia-Falgueras, 2009). Second, although studies that provided step-by-step molecular explanations were prioritized, relatively few sources were able to connect genetic, hormonal, epigenetic, and neurodevelopmental factors into a continuous explanatory model (Bakker, 2022; Kundakovic & Tickerhoof, 2024).

A significant limitation is the continued reliance on cisgender male and female comparison groups within much of the available literature. This pattern reflects not only methodological constraints, but also structural biases rooted in the epistemological dominance of cisgender and binary paradigms in biological research. Sex and gender are often conceptualized as dichotomous variables rather than expressions of a broader spectrum of human variation, limiting interpretive validity and narrowing how biological mechanisms are understood across identities. Consequently, many conclusions are extrapolated from cisgender samples rather than empirically validated with gender diverse participants.

While such studies remain essential for tracing molecular and neurodevelopmental pathways, their foundational assumptions shape both experimental design and theoretical interpretation. Future work should intentionally include gender-diverse participants and employ frameworks that move beyond cis-binary models to test whether the mechanisms identified here hold across populations. Moreover, the predominance of Western samples further restricts generalizability, as environmental and genetic variation may influence developmental and hormonal processes in ways not captured by current data. Expanding demographic and cultural representation would enhance both the validity and inclusiveness of future research.

Similarly, while human studies were prioritized, ethical constraints necessitated the inclusion of animal models, and translation across species introduces uncertainty (Bobotis et al., 2023; Nelson et al., 2019). Collectively, these studies illustrate how biological differentiation is multifactorial yet remains constrained by methodological binarism that limits generalizability to gender diverse populations. Together, these methodological reflections provide transparency about the scope.

The literature search process outlined in this chapter established a representative body of evidence spanning genetics, epigenetics, hormonal signalling, microglial activity, and prenatal environmental influences. Through iterative, concept-driven search strategies, the review identified 27 peer-reviewed studies that together provide a molecular and developmental foundation for understanding gendered neural differentiation. The inclusion and exclusion criteria ensured that the selected literature contributed directly to stepwise biological mechanisms rather than broader psychosocial explanations. The challenges and limitations described clarify the strengths and constraints of the evidence base, particularly the reliance on cisgender samples, disciplinary fragmentation, and inconsistent terminology. Building on this

curated body of research, Chapter 3 synthesizes these molecular and developmental findings into an integrative explanatory model of how biological processes contribute to gender diversity.

Chapter 3: Literature Review

Overview of the Research Topic

Gender diverse individuals are a growing population who face ongoing misunderstanding, stigma, discrimination, exclusion, and violence, contributing to higher rates of substance use and suicidality (Carone et al., 2021; Johns et al., 2019; Valentine & Shipherd, 2018). A common source of distress is the lack of understanding from both society and mental health professionals about who gender diverse people are and the biological factors that contribute to their identities (Bowers & Whitley, 2020). Recognizing that gender diversity is partly rooted in stable, measurable biological factors could foster greater acceptance and self-understanding (Bowers & Whitley, 2020). This capstone explores the biological factors underlying gender diversity with the goal of supporting gender diverse individuals and informing practitioners who can, in turn, share this knowledge.

Purpose and Structure of the Literature Review

This chapter reviews current research to elucidate the biological factors underlying gender diversity. It synthesizes research findings to explain how typical gendered differentiation could be altered, how those alterations affect microglia, the brain's neuronal architects, and how these processes together provide biological support for gender on a spectrum. The systems involved in gendered neurological differentiation (GND) are diverse, complex, interdependent, and vast. As such, this review focuses on select themes, genetics, epigenetics, hormones, signalling molecules, endocrine disruptors, and microglia to offer a coherent molecular explanation.

Themes in this review were identified through an inductive, iterative synthesis process, in which recurring biological mechanisms relevant to gendered neurodevelopment became apparent

through sustained engagement with the literature. As patterns emerged across independent sources, studies were grouped according to shared molecular, cellular, and developmental processes. This inductive process gave rise to the core thematic domains presented in Chapter 3, including genetics, epigenetics, hormones, microglia, signalling molecules, endocrine disruptors, and environmental influences.

Neurodevelopmental theory provided the conceptual structure for integrating and sequencing the inductively derived themes and subthemes across prenatal development. As recurring biological mechanisms, processes, and points of convergence became apparent through analysis of the included studies, these patterns were grouped into thematic domains and associated subthemes to capture meaningful distinctions within the literature. The framework guided how relationships among these biological processes were interpreted and organized developmentally, without determining the content of the themes or subthemes themselves. The following table outlines these themes and their sub-themes.

Table 2

Overview of Findings

Themes	**Sub-Themes**

1. Factors Contributing to Brain and Body Differentiating Separately	a. The critical period for gendered neurological differentiation b. Hormone timing for the differentiation of the body c. Signalling molecules
2. Genetics and Epigenetics of Gender Identity	a. Androgen and estrogen receptor polymorphisms b. Genetic and Epigenetic Markers Observed from Differences in Sexual Development (DSD) c. Pathways Leading to 46XYGD d. Genetics e. Epigenetics
3. Endocrine disruptors & Environmental Influences	a. Phytoestrogens b. Antiandrogenic compounds c. Pharmaceuticals
4. Microglia	a. Microglial migration b. Microglial Interplay with Steroid Hormones and Signalling Molecules

Factors Contributing to Brain and Body Differentiating Separately

During gendered neurological differentiation (GND), the brain and body undergo sex-related developmental processes that occur along partially distinct timelines (Bakker, 2022). During specific windows of fetal development, the brain may alter its developmental trajectory and physical organization in response to hormonal and molecular signals, sometimes independently of somatic sex differentiation (Bobotis et al., 2023; McCarthy et al., 2018; VanRyzin et al., 2020). When these processes diverge from typical patterns in which brain and body differentiate concordantly, the result may be a gender-diverse outcome (du Toit & Swart, 2021).

This theme is examined through subthemes addressing the timing of critical periods for brain versus body differentiation, the role of placental and fetal hormone signaling, and the influence of molecular messengers that mediate communication between developing systems.

The Critical Period for Gendered Neurological Differentiation

The critical period is the time in which a stimulus can alter a crucial developmental process (McCarthy et al., 2018). Critical periods have an opening and a closing. The opening is triggered by extrinsic factors such as hormones or intrinsic genetic expression, and the closing refers to when a specific stimulus can no longer alter development (McCarthy et al., 2018). The critical period for neurological sexual differentiation is defined as when the testes of XY individuals begin producing an abundance of androgens, and the closing occurs via epigenetic factors when XX individuals are no longer sensitive to masculinization in the presence of testosterone (McCarthy et al., 2018).

DNA methylation, one of the key epigenetic mechanisms regulating histones and gene expression, appears to be a central factor in these critical periods of GND (Nelson et al., 2019). This is known because altering the natural state of DNA methylation after the critical period has finished allows it to be reopened, permitting hormones to once again alter neural physiology (Nelson et al., 2019). For humans, this critical period occurs entirely during the fetus's time in the womb (Bakker, 2022; Swaab & Garcia-Falgueras, 2009).

While there is some overlap, there is a significant portion of development during which the brain is sexually differentiated separately from the body, starting during the eighth week of pregnancy, and lasting at least until the twenty-fourth week (Bakker, 2022; Swaab & Garcia-Falgueras, 2009). The closing of the critical window is not uniform across the brain as mammalian neurological structures have differing timings for their endpoints such as the

masculinization of the structures responsible for sexual behavior versus those involved in pituitary control (McCarthy et al., 2018). To understand the biological factors underlying gender diversity, it is essential to examine the role of hormones during these critical periods.

Because the brain's critical period spans a longer and later developmental window than the body's (Bakker, 2022; Swaab & Garcia-Falgueras, 2009), deviations in hormonal or epigenetic signaling can redirect neural trajectories while the body's differentiation remains unchanged (McCarthy et al., 2018; Nelson et al., 2019). This provides a concrete biological pathway through which brain and body can follow partially decoupled developmental routes, directly supporting gender diversity as an expected outcome of variation in gendered neurodevelopment rather than as a deviation from a fixed binary norm (Bakker, 2022; Nelson et al., 2019).

Hormone Timing for the Differentiation of the Body

From conception up until approximately 8 weeks, the maternal ovary is the main producer of hormones until it is joined by the maternal adrenal, the placenta, fetal gonads, and the fetus's adrenal gland, commonly called the fetal adrenal (du Toit & Swart, 2021). Once fetal hormone production is initiated, both the fetus and placenta are interconnected in regulating and producing hormones (du Toit & Swart, 2021).

For typical male somatic development, the testes are necessary to initiate the required androgen bursts, with testicular differentiation occurring around the sixth week of pregnancy following a cascade of gene activation centered on the sex determining region Y (SRY) gene on the Y chromosome (Bao & Swaab, 2011; Okashita & Tachibana, 2021; Swaab & Garcia-Falgueras, 2009). In this developmental context, androgens produced by the testes commonly drive differentiation of the body between approximately the sixth and twelfth weeks of

pregnancy (Bao & Swaab, 2011; Okashita & Tachibana, 2021; Swaab & Garcia-Falgueras, 2009). In the relative absence of androgen signaling during this window, ovarian development generally proceeds, and the fetus's body most often follows female-typical somatic differentiation pathways, which also occur between weeks 6 and 12 of gestation (Swaab & Garcia-Falgueras, 2009; Bao & Swaab, 2011).

To summarize this general overview of brain and body gendered differentiation, the body's critical period occurs earlier, spanning approximately weeks 6 to 12, while the critical period for the brain extends from week 8 to at least week 24 (Bakker, 2022; Bao & Swaab, 2011; Swaab & Garcia-Falgueras, 2009). In contrast to the brain's later and more extended critical period, the body completes its differentiation earlier, closing its window before the brain remains sensitive to shifting hormonal and epigenetic influences (Bakker, 2022; Nelson et al., 2019). This developmental asymmetry helps explain how gender diverse identities can emerge even when bodily development follows typical male–female patterns, challenging models that equate gender solely with visible anatomy and supporting spectrum-based accounts of gender development (McCarthy et al., 2018; Okashita & Tachibana, 2021; Swaab & Garcia-Falgueras, 2009). Across these critical windows, hormonal, genetic, and epigenetic processes shape both bodily and neural differentiation, and this paper examines how variation in these biological factors can arise during fetal development (Bao & Swaab, 2011; Kundakovic & Tickerhoof, 2024; Okashita & Tachibana, 2021; Santoro et al., 2021; Swaab & Garcia-Falgueras, 2009).

Signalling Molecules

Hormones. Hormones play a critical role in sex differences during neurodevelopment, and as hydrophobic molecules, sex hormones pass through the blood-brain barrier and influence the differentiation of the brain (Nelson et al., 2019). Although the expression of sex determining

and differentiating genes shows sexual dimorphism, estrogen receptors are expressed in both male and female embryos throughout development (Bobotis et al., 2023). These receptors respond to hormonal signals from the gonads and surrounding tissues, guiding sexual and physiological differentiation across multiple systems in a time-dependent manner (Nelson et al., 2019). A lack of androgens and elevated estradiol production will result in GND that trends toward the female end of the spectrum (Bakker, 2022; McCarthy et al., 2018; Swaab & Garcia-Falgueras, 2009).

Cortisol also contributes to female differentiation, peaking at 8–9 weeks of gestation and suppressing fetal androgen production (du Toit & Swart, 2021). Altering levels of androgens or estrogens, or disrupting their effects during brain differentiation, has been shown to significantly influence gendered neurodevelopment (Bakker, 2022; Fernández et al., 2018). Relatedly, cyclical fluctuations in estrogen, such as those occurring across the estrous cycle, have been found to affect emotion regulation, metabolic processes, reward pathways, and memory formation, underscoring the hormone's broad influence on brain and behavior (Kundakovic & Tickerhoof, 2024).

Together, these findings demonstrate that hormone-driven neurodevelopment operates through graded, time-sensitive pathways rather than producing fixed outcomes, providing a biological basis for gender diversity and helping to correct binary assumptions that contribute to misunderstanding and invalidation in clinical settings (Bakker, 2022; Nelson et al., 2019).

Endocannabinoids. The endocannabinoid system (ECS), crucial for pain regulation and neurodevelopment, also interacts with hormonal systems (Santoro et al., 2021). The ECS comprises endocannabinoids, cannabinoid receptors (CB1 and CB2), and enzymes that synthesize and degrade endocannabinoids (Blanton et al., 2021). The presence of

endocannabinoid receptors and their signalling in neural progenitors as well as microglia, and their interactions with sex hormones, suggests the ECS significantly influences gendered neurodevelopment (Blanton et al., 2021; Krebs-Kraft et al., 2010; Rezende et al., 2023).

While both are present in our neural cells, CB1 is more abundant in the central nervous system, while CB2 is more prominent in glial cells, including microglia (Blanton et al., 2021; Rezende et al., 2023). The endocannabinoid 2-arachidonoylglycerol (2-AG) and its catabolic enzyme monoacylglycerol lipase (MAGL) are implicated in sexual differentiation (Blanton et al., 2021; Krebs-Kraft et al., 2010). Estrogen can reduce MAGL expression, leading to increased amounts of 2-AG, as evidenced by the estrogen-mediated increase in 2-AG levels in the female pituitary and hypothalamus (Blanton et al., 2021; Krebs-Kraft et al., 2010). This is a two-way street as endocannabinoids have also shown the ability to regulate the metabolic enzymes of sex hormones (Santoro et al., 2020).

Cannabinoid receptors are active early in brain development, influencing neurogenesis, neuronal differentiation, and synaptogenesis (Krebs-Kraft et al., 2010). Neural progenitor cells express CB1 and CB2 and produce endocannabinoids, highlighting their neurodevelopmental significance (Krebs-Kraft et al., 2010). ECS components are also found in hormone regulating brain regions, with sex hormones modulating cannabinoid receptor density, affinity, and efficacy, especially in females (Blanton et al., 2021). CB1 shows sex-specific distribution in the hypothalamus, pituitary, cortex, amygdala, mesencephalon, and pain-processing areas (Blanton et al., 2021).

These findings suggest that the endocannabinoid system contributes an additional layer of biological variability to gendered neurodevelopment by shaping neural differentiation through hormone endocannabinoid system crosstalk (Blanton et al., 2021; Krebs-Kraft et al., 2010). This

complicates models that view gender development as primarily hormone-driven and instead supports a multisystem explanation in which diverse neurochemical pathways can shift gendered developmental trajectories. Disseminating this knowledge to mental healthcare providers may improve care for gender-diverse individuals by addressing informational gaps that currently hinder supportive practice (Carone et al., 2021; Johns et al., 2019; Santoro et al., 2021; Valentine & Shipherd, 2018).

Genetics and Epigenetics of Gender Identity

To better comprehend the biological basis of gender identity, this section will explore how genetic factors, such as receptor polymorphisms and mutations associated with differences in sexual development (DSD), together with epigenetic factors such as chromatin remodeling, can influence gendered development during critical prenatal periods.

Androgen and estrogen receptor polymorphisms (DNA sequence variations)

Androgens and estrogens are critical factors in GND and the body's sexual differentiation with receptor polymorphisms potentially altering their effects (Fernández et al., 2018). Three steroid hormone receptors that have been implicated in GND are the androgen receptor (AR), estrogen receptor alpha (ERa), and estrogen receptor beta (ERb) (Fernández et al., 2018; VanRyzin et al., 2020). These receptors are expressed in human neurons, astrocytes, and microglia across all human developmental stages and upon binding with their respective hormones, function as transcription factors, regulating gene activation and RNA production (Fernández et al., 2018; Nelson et al., 2019; Santoro et al., 2021; VanRyzin et al., 2020).

Fernández and colleagues (2018) examined polymorphisms for AR, ERa, and ERb and found there were significant allele differences for transgender men and women when compared to each other and their cisgender counterparts. In cisgender individuals, the AR allele has repeats

of cytosine, adenine, and guanine (CAG). In cisgender individuals, the ERb allele repeats of cytosine and adenine (CA) (Fernández et al., 2018). For transgender women, in some cases, their AR alleles contain more CAG repeats, which is paired with an ERb polymorphism that has short alleles with fewer CA repeats (Fernández et al., 2018). In other cases there were long alleles for ERb with more CA repeats, paired with short alleles for AR containing less CAG repeats (Fernández et al., 2018). Having either of these AR or ERb polymorphisms alone was not sufficient to correlate with differentiating as a trans woman (Fernández et al., 2018).

For ERa, having two A alleles was associated with differentiating as a transgender male, while having one A allele and one G allele was associated with differentiating as a cisgender woman (Fernández et al., 2018). The A allele refers to a single nucleotide polymorphism where the researchers checked for the presence of either adenine (A) or guanine (G) in the ERa allele (Fernández et al., 2018). Fernández and colleagues (2018) observed in ERb a direct correlation between the number of CA repeats in the ERb gene and differentiating as a transgender man, as well as discovering that transgender men and women shared the same estrogen receptors polymorphisms.

An example of how these polymorphisms affect GND can be found in the differing base pairs or repeats on these alleles in the ESR1 gene, which produces ERa, affecting how well transcription factors can bind to DNA to regulate expression (Fernández et al., 2020). Fernández and colleagues (2020) suggest that these differences in gene expression can alter important properties of the ERa receptor, affecting its response to estrogens during the critical period of GND in the womb.

These polymorphisms can influence GND by altering receptor binding efficacy during critical prenatal periods where, for example, variations in the ESR1 gene, which encodes ERα,

can affect transcription factor binding and receptor sensitivity to estrogen (Fernández et al., 2020). Taken together, these findings demonstrate that variation in receptor structure and binding efficiency can meaningfully alter neural sensitivity to sex hormones during critical developmental windows, introducing biologically grounded variability in gendered neurodevelopment independent of circulating hormone levels. (Fernández et al., 2020; Santoro et al., 2021; Spencer et al., 2021). Additionally, differences in sexual development (DSD) offer further insights into the genetic basis of gender diversity (Blanton et al., 2021; Kundakovic & Tickerhoof, 2024; Okashita & Tachibana, 2021).

Genetic and Epigenetic Markers Observed from Differences in Sexual Development

Differences in sexual development are conditions that result in atypical sexual development beyond the male-female binary, often mediated by genetic and epigenetic components (Kundakovic & Tickerhoof, 2024; Okashita & Tachibana, 2021). These genetic and epigenetic alterations can result in significant downstream GND effects (Kundakovic & Tickerhoof, 2024; VanRyzin et al., 2020).

One such condition, 46XY gonadal dysgenesis (46XYGD), arises from disruptions or delays in SRY-related pathways (Okashita & Tachibana, 2021). Individuals with 46XYGD may present with female, blended, or underdeveloped male reproductive structures, often with low or absent testosterone production (Elzaiat et al., 2022; Okashita & Tachibana, 2021).

Pathways Leading to 46XYGD

Mutations in the androgen receptor (AR) gene are one pathway by which 46XYGD can occur leading to androgen insensitivity syndrome (AIS) (Davey & Grossmann, 2016). 46XYGD occurs on a spectrum, where AR mutations that allow limited androgen binding, resulting in blended genital development including a small phallus and partially fused labial/scrotal folds

(Davey & Grossmann, 2016). Over 400 AR genetic mutations have been identified that can result in a receptor with reduced binding affinity (Davey & Grossmann, 2016). In contrast, complete AIS (CAIS) eliminates androgen binding, producing fully female or blended genitalia (Elzaiat et al., 2022). Additionally, enzyme deficits in hormone biosynthesis pathways required for male genital formation can contribute to 46XYGD (Elzaiat et al., 2022). These pathways demonstrate how variation in androgen receptor function and hormone synthesis operate as biological factors that can produce spectrum based developmental outcomes relevant to biological contributions to gender diversity.

Genetics

Genetic analysis shows that most 46XYGD individuals have mutations in the high mobility group (HMG) box, the DNA region to which the SRY protein binds to initiate transcription (Okashita & Tachibana, 2021). The transcription factor Nr5a1 regulates SRY activation and impacts other sexual differentiation elements, including ovary development genes and chromobox homolog 2 (CBX2) (Elzaiat et al., 2022; Okashita & Tachibana, 2021). CBX2 mutations reduce the expression of Nr5a1 and GATA-4, thereby limiting SRY activation and resulting in XY sex reversal. Since CBX2 also epigenetically suppresses ovary development, mutations to CBX2 further inhibit testes development (Okashita & Tachibana, 2021).

Other mutations in the SRY system implicated in partial to total male to female reversal include, but are not limited to, the MAPK31, SRY, and SOX genes, the stress response gene GADD45G, and transcription factor WT1 (Elzaiat et al., 2022; Okashita & Tachibana, 2021). These genetic variations can alter transcription factor binding, hormone release timing, and the production of essential proteins and enzymes, ultimately influencing gendered neurodevelopment (Elzaiat et al., 2022; Kundakovic & Tickerhoof, 2024; Okashita & Tachibana, 2021).

Collectively, these findings identify genetic variation and receptor polymorphisms as biological factors that can modulate hormone sensitivity and developmental timing during prenatal critical periods, contributing to biologically grounded variability in gendered neurodevelopment.

Epigenetics

Epigenetics involves alterations to chromatin that affect gene expression without altering the DNA sequence (McCarthy et al., 2018). Chromatin, composed of DNA and predominantly histone proteins, is part of our epigenetic regulation system (Kundakovic & Tickerhoof, 2024). The base unit of this epigenetic system is the nucleosome where DNA is wound around eight histones (Kundakovic & Tickerhoof, 2024; McCarthy et al., 2018; Okashita & Tachibana, 2021). The degree of DNA accessibility to transcription factors depends on how tightly it is wound around these histones (Kundakovic & Tickerhoof, 2024; McCarthy et al., 2018).

The tightness is modulated by proteins that acetylate or methylate the histones (Lorch et al., 2023). Histone acetyltransferases (HATs) add acetyl groups to lysine residues on histones, neutralizing their positive charge and loosening DNA which allows more access (Kundakovic & Tickerhoof, 2024). Conversely, histone deacetylases (HDACs) remove acetyl groups, tightening DNA around histones by restoring the positive charge (Okashita & Tachibana, 2021). However, chromatin tightness spans a continuum rather than having open/closed or on/off states (Lorch et al., 2023; Okashita & Tachibana, 2021). Fine-tuning occurs due to each nucleosome containing approximately 80 lysine residues, 10 per histone, that can have up to three methyl groups attached, though only about 20 are typically available for modification, allowing variable levels of DNA accessibility (Kundakovic & Tickerhoof, 2024; Lorch et al., 2023).

For example, in 46XYGD, when one of two HATs associated with regulating the SRY system, p300 and CBP, have deficiencies, it leads to partial male to female sex reversal while

deficiencies in both lead to a complete reversal (McCarthy et al., 2018; Okashita & Tachibana, 2021). This illustrates how epigenetics, via changes in histone acetylation and methylation can also be a biological factor affecting gender diversity through their ability to alter the expression of the genes that play a crucial role in the differentiation of human bodies (Kundakovic & Tickerhoof, 2024; Lorch et al., 2023; McCarthy et al., 2018). In the case of 46XYGD, this leads to altered testosterone production that affects downstream neurodevelopment (Kundakovic & Tickerhoof, 2024; Lorch et al., 2023; McCarthy et al., 2018). Together, this body of evidence demonstrates that epigenetic regulation functions as a set of biological factors capable of modulating gene expression and hormone production during prenatal critical periods, providing a mechanistic pathway through which biologically grounded variation in gendered development, including gender diversity, can arise.

The information presented regarding receptor polymorphisms, differences in sexual development, and epigenetic regulation demonstrates how multiple biological factors, acting independently or in combination during prenatal critical periods, can influence gendered development through mechanisms such as graded changes in chromatin accessibility that fine tune gene expression across developmental pathways (Fernández et al., 2018; Fernández et al., 2020; Kundakovic & Tickerhoof, 2024; Okashita & Tachibana, 2021; Santoro et al., 2021). The potential impacts of genetics and epigenetics on gendered development, could be further amplified or reduced by endocrine disruptors.

Endocrine Disruptors & Environmental Influences

Endocrine disrupting chemicals (EDCs) are a biological factor that can affect hormone levels and effectiveness during critical periods of development relevant to gender (Gore et al., 2014). This section will explore the relationship between EDCs, hormones, epigenetic regulation,

gene expression, receptor binding, and cellular signalling as it relates to gender diversity (Gore et al., 2014; Santoro et al., 2021; Solleiro-Villavicencio et al., 2020).

Phytoestrogens

Bisphenol A (BPA). BPA is a phytoestrogen, a compound capable of mimicking or altering estrogen levels, that is found in plasticizers, parabens, and pesticides (Santoro et al., 2021). Bisphenol A (BPA), a widespread environmental toxin found in food and drink packaging, is detected in 93% of Americans over age 6 and can cross both the placental and blood-brain barriers (Gore et al., 2014; Santoro et al., 2021). In humans, BPAs have been able to affect the endocannabinoid systems of pregnant women, potentially influencing developing fetuses (Santoro et al., 2021). For example, they can disrupt sex specific cognitive patterns, such as male spatial learning, and alters estrogen receptor expression, downregulating ERb and increasing methylation of ERa genes (Solleiro-Villavicencio et al., 2020). BPA exposure in rodents has led to astrocytosis via ERa dysregulation (Santoro et al., 2021). Even low levels of EDCs may exert compounded effects due to combined exposures (Gore et al., 2014).

BPA has been shown to interfere with our endocrine systems differentially based on gender such as disruptions to the typical gendered pattern of male spatial learning abilities (Solleiro-Villavicencio et al., 2020). At the molecular level, BPA has been shown to reduce ERb expression and increase DNA methylation of the gene encoding ERa (Solleiro-Villavicencio et al., 2020). Even low environmental levels of EDCs may exert temporally compounded effects due to combined exposures (Gore et al., 2014). BPA's ability to alter hormone receptor expression and epigenetic states during critical windows demonstrates that gendered neurodevelopment is sensitive to environmental phytoestrogenic inputs (Gore et al., 2014; Santoro et al., 2021). This evidence challenges deterministic models of gender development

(Spencer et al., 2021) by showing how external exposures can nudge neural differentiation in ways that align with spectrum-based accounts of gender diversity (Solleiro-Villavicencio et al., 2020).

Soy Isoflavones and Diethylstilbestrol. Exposure to environmental estrogens such as soy isoflavones and the synthetic estrogen Diethylstilbestrol may reduce estrogen production by the placenta and lower fetal adrenal cortisol levels (Kaludjerovic & Ward, 2012). Cortisol stimulates the release of dehydroepiandrosterone (DHEA), an estrogen precursor, from the fetal adrenal cortex, which in turn promotes placental estrogen production (Kaludjerovic & Ward, 2012). Environmental estrogens can disrupt this process by reducing DHEA production, thereby decreasing estrogen availability for fetal development and differentiation (Kaludjerovic & Ward, 2012). These findings identify alterations in placental and fetal steroid synthesis as biologically relevant factors that modify the hormonal context of development, demonstrating how prenatal biochemical environments contribute additional variability to gendered brain differentiation.

Antiandrogenic Compounds

Di-(2-ethylhexyl) phthalate (DEHP) an antiandrogenic EDC, lowers testosterone, and during pregnancy can alter hippocampal neuron morphology, increase anxious behaviors, and impair spatial memory, particularly in male rodents (Solleiro-Villavicencio et al., 2020). Di-n-butyl phthalate (DBP), another antiandrogenic EDC, can also alter fetal hippocampal morphology resulting in poor reflexes, and lower memory retention (Solleiro-Villavicencio et al., 2020). These effects were transgenerational, being epigenetically passed on to two subsequent generations (Solleiro-Villavicencio et al., 2020).

These antiandrogenic effects illustrate how reduced androgen signaling can shift masculinization trajectories at the molecular and cellular levels, producing long term and even

transgenerational changes to neural differentiation (Solleiro-Villavicencio et al., 2020). Such findings extend existing theories by showing that gendered outcomes emerge from variable androgenic environments rather than fixed, binary developmental paths (Spencer et al., 2021).

Pharmaceuticals

Pharmaceuticals containing phytocannabinoids like cannabidiol (CBD) and Delta-9-tetrahydrocannabinol (THC), have shown the ability to alter the natural functioning of our endocannabinoid system (Rezende et al., 2023). CBD and THC can cross the placental and blood-brain barriers to activate the CB1 and CB2 receptors, potentially contributing to the spectrum of GND (Santoro et al., 2021). Dexamethasone, a synthetic glucocorticoid, influences fetal microglia development in a sex dependent manner by interacting with steroid hormones produced by the mother and the fetal adrenal cortex (Bobotis et al., 2023; VanRyzin et al., 2020). This results in enlarged microglia in the female hippocampus and male nucleus accumbens (Bobotis et al., 2023; VanRyzin et al., 2020).

The evidence provided on phytoestrogens, antiandrogens, and pharmaceuticals show how EDCs can influence gendered differentiation by altering hormone signalling, receptor function, neurodevelopmental cell morphology and epigenetic regulation, potentially across generations (Bobotis et al., 2023; Gore et al., 2014; Santoro et al., 2021; Solleiro-Villavicencio et al., 2020; VanRyzin et al., 2020). This demonstrates the need to incorporate environmental biology into a clinician's model of gender diversity to help counter harmful approaches (e.g., social contagion, and transnormative theories) and support affirming, spectrum-based frameworks such as GALA (Abreu et al., 2022; MacKinnon et al., 2025; Spencer et al., 2021).

Microglia

Microglia act as neurological immune cells playing a significant role in brain development and maintaining homeostasis during inflammation (VanRyzin et al., 2020). Their membranes contain numerous receptors known as the microglial sensome, which includes receptors for neurotransmitters, neuromodulators, and steroid hormones (Bobotis et al., 2023; Nelson et al., 2019). This includes AR, ERa, ERb, and progesterone receptors (PR), and androgen and progesterone receptors, which are highly expressed during neurodevelopment in the womb (Bobotis et al., 2023; VanRyzin et al., 2020). However, the expression of these hormone receptors will differ based on their location in the brain (VanRyzin et al., 2020).

Microglia are highly responsive to environmental changes, influencing neurological development (Bobotis et al., 2023; Nelson et al., 2019; VanRyzin et al., 2020). Their involvement in neurological development includes influencing cell proliferation, phagocytosis, synaptic pruning, and cellular maintenance, often in sexually differentiated brain regions, positioning them as both targets and agents of sexual differentiation (VanRyzin et al., 2020). This section will examine the interplay between microglial migration, interactions with signalling molecules, and their effects on GND (Bobotis et al., 2023; Bordt et al., 2020; VanRyzin et al., 2020).

Microglial Migration

In both humans and rats, microglia colonize the brain from the embryonic yolk sac in waves, coinciding with vascular development and occurring before the blood brain barrier closes (Bobotis et al., 2023; Bordt et al., 2020; VanRyzin et al., 2020). Microglia are detectable in humans as early as 4.5 weeks of gestation, preceding other neural cells such as neurons, astrocytes, and oligodendrocytes, underscoring their foundational influence on neurodevelopment (Bordt et al., 2020; Nelson et al., 2019; VanRyzin et al., 2020). Differences in the timing and pathways of microglial arrival may contribute to gendered neurodevelopment, for

example, the gender based cerebral blood flow differences observed postnatally, which shift during adolescence and diminish in later adulthood, may also have prenatal counterparts that shape early microglial distribution (Bordt et al., 2020).

Hormonal and vascular factors further shape these trajectories. Estrogen modulates vascular endothelial growth factor (VEGF), which governs blood vessel formation between the yolk sac and the developing brain (Aberdeen et al., 2024; Bordt et al., 2020). Elevated estrogen levels increase VEGF and promote vascular growth, while reduced estrogen levels diminish vessel formation and may delay microglial arrival, shifting the timing of their interactions with neural progenitors (Aberdeen et al., 2024; Bordt et al., 2020). Because microglia respond dynamically to the local molecular cues present at the time they enter a region, even small shifts in arrival timing can expose them to different microenvironments, altering their activation state, pruning behavior, and downstream contributions to neural differentiation (VanRyzin et al., 2020).

Taken together, these migration dynamics highlight how developmental timing, vascular pathways, and hormonal environments jointly shape region-specific microglial presence (Aberdeen et al., 2024; Bordt et al., 2020). This reinforces the idea that gendered neurodevelopment emerges from complex, interacting processes rather than a uniform, binary sequence (Spencer et al., 2021; VanRyzin et al., 2020).

Microglial Interplay with Steroid Hormones and Signalling Molecules

Microglial responses to steroid hormones are region-specific, varying with the local microenvironment and developmental stage (VanRyzin et al., 2020). These responses are limited to critical periods: prenatal development, mini puberty, and puberty. During these windows, hormones influence microglial morphology, density, activation state, and gene/protein expression (Bobotis et al., 2023; VanRyzin et al., 2020).

Transcriptome studies reveal temporally distinct gene expression phases in microglia, aligning with the prenatal gonadal hormone surge and neural differentiation (Bordt et al., 2020). In the hippocampus, 1109 genes were found to be differentially expressed between male and female microglia (Bobotis et al., 2023), indicating a clear temporal and regional component to sex specific gene regulation.

For example, estrogen treatment in newborn female rats was able to alter the number of microglia in a phagocytic state to male levels in the amygdala (Bobotis et al., 2023) where during the prenatal androgen surge, male rats have more microglia in a phagocytic state in the amygdala (VanRyzin et al., 2020). These examples illustrate the regionally and temporally specific nature of hormone microglia interactions, reinforcing the sexually differentiated impact on brain development (Bobotis et al., 2023; Nelson et al., 2019; VanRyzin et al., 2020).

Neuronal signalling is also implicated in GND, as microglia express the IL1RL1 receptor which interacts with neuron derived interleukin-33 (IL33), increasing phagocytic activity (Martinez Ramirez et al., 2023). Neuronal CB1 receptors inhibit IL-33 release, while microglial CB2 receptors regulate IL1RL1 expression. These processes are influenced by 2-AG levels, with CB1 and CB2 signaling jointly suppressing microglial phagocytosis (Martinez Ramirez et al., 2023).

When combined, the evidence on microglial migration, region and hormone-specific responses, and interactions with neurotransmitter and endocannabinoid systems shows microglia as critical biological factors in gendered neurodevelopment (Blanton et al., 2021; Kundakovic & Tickerhoof, 2024; Santoro et al., 2021; Spencer et al., 2021; VanRyzin et al., 2020). This highlights biology as a contributing factor to gender diversity and seems to support the need for individualized counselling interventions that align with GALA's gender affirming approach of

moving beyond the binary (Blanton et al., 2021; Kundakovic & Tickerhoof, 2024; Okashita & Tachibana, 2021; Santoro et al., 2021; Spencer et al., 2021; VanRyzin et al., 2020).

Discussion

The critical period for gendered neurodevelopment during weeks 8 to 24 of gestation is shaped by multiple biological factors including signalling molecules, genetics, epigenetics, microglia and environmental agents (Bakker, 2022; Bao & Swaab, 2011; Okashita & Tachibana, 2021; Swaab & Garcia-Falgueras, 2009; VanRyzin et al., 2020). Altering these factors after the critical period of the body's differentiation may lead to brain structures diverging from the chromosomal sex by altering microglial instructions to cull, proliferate, maintain, or prune neurons in regionally dependent ways, between and within genders (Bakker, 2022; Okashita & Tachibana, 2021; Solleiro-Villavicencio et al., 2020; VanRyzin et al., 2020).

Microglial Driven GND Modulation and Evidence for a Gender Spectrum

A neurological gender spectrum is supported by the multiple independent, and often interconnected, nodes capable of altering microglial behavior. Microglia express ERa, ERb, and AR making them sensitive to known receptor polymorphisms (Fernández et al., 2018; Nelson et al., 2019; Santoro et al., 2021). AR alone has over 400 known mutations, each variably reducing androgen binding affinity, which can alter microglial behavior (Bobotis et al., 2023; Nelson et al., 2019; Okashita & Tachibana, 2021). Mutations affecting the SRY gene regulation can also reduce androgen production, further altering microglial signalling in XY individuals (Nelson et al., 2019; Okashita & Tachibana, 2021; VanRyzin et al., 2020).

Endocrine disruptors can influence estrogens, androgens, endocannabinoids, and cortisol, each of which may influence microglial-driven GND (Blanton et al., 2021; Santoro et al., 2021; Solleiro-Villavicencio et al., 2020). Phytoestrogenic BPA and antiandrogenic DEHP, can alter the

levels and functioning of sex hormones, soy isoflavones and synthetic estrogens can alter cortisol levels, and BPAs, CBD and THC can alter endocannabinoid function (Blanton et al., 2021; Kaludjerovic & Ward, 2012; Santoro et al., 2021). The spectrum is further supported by the wide variability in individual exposure to endocrine disruptors, both in type and dosage (Blanton et al., 2021; Chioccarelli et al., 2021; VanRyzin et al., 2020).

BPA has been shown to reduce MAGL, the enzyme that degrades 2-AG, thereby increasing CB2 receptor expression (Chioccarelli et al., 2021). Elevated 2-AG increases CB2 signaling which suppresses microglial phagocytosis (Blanton et al., 2021; VanRyzin et al., 2020). Similarly, cannabinoids such as CBD and THC can cross the placental and blood–brain barriers, activate CB1/CB2 receptors, and reduce microglial phagocytosis (Blanton et al., 2021; Santoro et al., 2021). For example, a reduction in phagocytosis could cause an XY individual's amygdala to shift toward a more female configuration (Blanton et al., 2021; Santoro et al., 2021).

CB1 and CB2 signalling also inhibit IL-33 and its receptor IL1-RL1, key drivers of microglial phagocytosis (Martinez Ramirez et al., 2023). This relationship is bidirectional as elevated MAGL and 2-AG levels suppress sex hormone levels, which further upregulate CB1/CB2 receptors, decreasing phagocytosis (Martinez Ramirez et al., 2023; Santoro et al., 2021). These interactions among IL-33, IL1-RL1, CB1, CB2, MAGL, 2-AG, estrogens, phytoestrogens, and microglial activity are illustrated in Appendix C. As with AR, ERα, and ERβ, polymorphisms in endocannabinoid, cortisol, and IL1-RL1 receptors may further contribute to the gender spectrum (Fernández et al., 2020). In all systems involved in gendered neurological development that rely on DNA and its gene products, epigenetics adds another layer to the gender spectrum through the continuum of chromatin accessibility (McCarthy et al., 2018; Okashita & Tachibana, 2021). This is illustrated by the histone acetyltransferase (HAT)

deficiencies in p300 and CBP in 46XY gonadal dysgenesis (McCarthy et al., 2018; Okashita & Tachibana, 2021). The extent of epigenetic influence depends on how many of the typically 20 lysine residues are affected (Kundakovic & Tickerhoof, 2024; Lorch et al., 2023).

Summary of the Literature Review

Human brains, including those of cisgender individuals, reflect mosaic patterns of male- and female-associated characteristics shaped by interacting genetic, epigenetic, maternal, and environmental influences, as well as microglial microenvironments within specific brain regions (VanRyzin et al., 2020). This chapter highlights three key takeaways from the reviewed literature that gendered neurodevelopment reflects mosaic brain organization, is shaped by multiple interacting biological systems, and produces a spectrum of diverse gendered neurodevelopmental outcomes that cannot be inferred from bodily differentiation alone. Across the evidence, gender-differentiated brain regions utilize distinct biological mechanisms and signaling dynamics, which together help explain why gender-diverse individuals often exhibit unique brain phenotypes rather than patterns aligning with either sex assigned at birth or binary gender categories (Blanton et al., 2021; Martinez Ramirez et al., 2023; Mueller et al., 2021; VanRyzin et al., 2020).

These biological themes form the foundation for Chapter 4, which translates this evidence into counselling applications. Understanding how multiple interacting biological factors can shape gendered neurodevelopment in ways that diverge from bodily differentiation provides counsellors with concrete tools for case conceptualization, psychoeducation, and advocacy within systems that often misunderstand gender diversity (Bakker, 2022; Bao & Swaab, 2011; Bowers & Whitley, 2020; Carone et al., 2021; Santoro et al., 2021; Swaab & Garcia-Falgueras, 2009; VanRyzin et al., 2020).

Gaps in the Research

As outlined in Chapter 2, much of the evidence synthesized in this paper derives from a combination of human studies and animal models, reflecting ethical constraints that limit experimental investigation of human neurodevelopment. To varying degrees, a key limitation across the themes of this paper is the lack of direct human studies, mainly due to the inability to perform invasive procedures on the human brain or expose individuals to harmful, life-altering substances (Bobotis et al., 2023; Gore et al., 2014). While animal testing itself contains ethical grey zones, it has been a vital tool for researchers since the 18th century to improve our understanding of the human body (Andersen & Winter, 2019). While findings from animal studies are not always directly translatable to humans, they often serve as critical starting points for identifying human-specific mechanisms (Andersen & Winter, 2019). In areas where human data is lacking, such models offer our best current insight into the biological underpinnings of gender diversity (Andersen & Winter, 2019; Bobotis et al., 2023).

Additionally, many microglia studies focus on gender differences between cisgender individuals, and papers with gender diverse participants largely exclude identities outside of the binary. No research reviewed included intersectional identities such as ethnicity, religion, neurodivergence, or disability. Expanding the scope of study to include these populations would yield more comprehensive and representative findings.

Ethical Considerations

According to the Tri-Council Policy Statement 2 no group should be unjustly excluded from the benefits of research. However, nonbinary and gender-diverse individuals with intersecting identities are underrepresented in the field of neurodevelopmental gender research (Canadian Institutes of Health Research, Natural Sciences and Engineering Research Council of Canada, & Social Sciences and Humanities Research Council, 2022). This exclusion violates the

ethical principle of respecting the dignity of persons and peoples, which advocates for fair and inclusive research practices (Canadian Psychological Association [CPA], 2017).

While endocrine disruptors and environmental factors can affect the biological factors underlying gender diversity, it is critical to emphasize that gender diversity does not stem from a single cause (Okashita & Tachibana, 2021; Solleiro-Villavicencio et al., 2020). Instead, it results from the interplay of numerous neurodevelopmental factors—such as genetics, epigenetics, microglia, hormones, neurotransmitters, signalling molecules, and cell receptors—alongside complex environmental interactions (Bordt et al., 2020; Lorch et al., 2023; Nelson et al., 2019; Okashita & Tachibana, 2021; Solleiro-Villavicencio et al., 2020; VanRyzin et al., 2020).

Given today's politicized climate surrounding gender diverse individuals, it is crucial to reject narratives suggesting gender diversity is entirely caused by environmental contaminants, as such claims can harmfully imply preventability or pathology (Abreu et al., 2022; Bowers & Whitley, 2020; Solleiro-Villavicencio et al., 2020; VanRyzin et al., 2020). Upholding ethical principle two, responsible caring includes the practitioner's responsibility to minimize harm by preventing the misinterpretation or misuse of scientific findings (CPA, 2017).

As the gatekeepers of gender affirming care, this is especially important for medical and mental health professionals, many of whom continue to adhere to a medical model that demands measurable traits to justify treatment (MacKinnon, 2018; MacKinnon et al., 2025). Biological factors are only one part of gender identity, and requiring specific biological markers to access care could result in significant harm (MacKinnon, 2018; MacKinnon et al., 2025). Reducing unnecessary gatekeeping aligns with principle one of respecting the dignity of people and persons and non-discrimination in helping those seeking life-saving care (CPA, 2017).

Chapter 4: Application to Clinical Practice

Introduction

The preceding chapters established the biological and neurodevelopmental foundations of gender diversity. Chapter 3, following the iterative, cross-disciplinary method established in Chapter 2, traced gender related developmental diversity to coordinated genetic, epigenetic, hormonal, microglial, and environmental influences during prenatal critical periods (Bakker, 2022; Okashita & Tachibana, 2021; Solleiro-Villavicencio et al., 2020; VanRyzin et al., 2020). The purpose of this chapter is to translate those findings into practical applications for counselling practitioners. Integrating biological knowledge into counselling psychology enhances affirming practice by improving case conceptualization, psychoeducation, and advocacy (Spencer et al., 2021). Doing so fulfills both ethical and professional responsibilities under the CPA code of ethics and the CAP standards of practice, which emphasize respect for dignity, responsible caring, and competent, inclusive, evidence-informed service delivery (CAP, 2023; CPA, 2017). This chapter outlines how biological understanding can guide everyday practice and provides specific recommendations for practice, supervision, and professional training.

Integrating Biological Knowledge into Counselling Practice

Case Conceptualization

As reviewed in Chapter 2, the inclusion criteria prioritized mechanisms with clear developmental relevance, including receptor polymorphisms (Fernández et al., 2020) and critical period timing (McCarthy et al., 2018). These mechanisms support strengths-based conceptualizations by showing that diversity in identity parallels and conforms with the natural diversity of developmental pathways, both emerging from the same layered biological processes

that contribute to the range of perspectives through which communities thrive (Kamalumpundi et al., 2024). Within counselling practice, this foundation clarifies why biological variation provides a meaningful context for understanding gendered experience and guides counsellors in applying these findings in case formulation, psychoeducation, and advocacy.

Knowledge of gendered neurodevelopment enables counsellors to conceptualize part of gender identity as an outcome of complex biological processes rather than as pathology or social deviation. Chapter 3 highlighted that brain and body differentiation operate on partially distinct timelines (Bakker, 2022; Bao & Swaab, 2011; Swaab & Garcia-Falgueras, 2009). Bringing this into case conceptualization helps practitioners contextualize why a client's affirmed gender may diverge from their gender assigned at birth.

Counsellors can use this perspective to reinterpret clients' narratives in ways that foster self-understanding and agency and frame gender identity within a strengths-based, developmental model rather than a deviation from the norm (Kamalumpundi et al., 2024; MacKinnon, 2018). This reorientation moves the client away from self-blame and toward an appreciation of developmental diversity as a natural expression of human variation. Such reframing aligns with the CPA Code of Ethics's respect for the dignity of people and persons, which emphasizes self-determination and the promotion of social justice (CPA, 2017).

Diversity at the molecular level parallels the broader value of diversity recognized in science and healthcare (Kamalumpundi et al., 2024). Research across STEMM disciplines shows that varied perspectives strengthen creativity, innovation, and problem solving, while a provider workforce that reflects the diversity of the communities it serves enhances access, trust, and overall outcomes (Kamalumpundi et al., 2024). Counsellors who recognize these parallels can approach gender diversity as a source of cognitive and relational strength (Bowers & Whitley,

2020; Spencer et al., 2021). The same variation that fosters innovation in scientific and organizational contexts also enriches counselling relationships by widening the range of perspectives, empathic responses, and strategies available within the therapeutic process (Kamalumpundi et al., 2024). Within a strengths-based framework, gender diversity thus becomes an asset that contributes to adaptability, creativity, and resilience in both individuals and the systems that support them.

Psychoeducation and Communication

Chapter 2 highlighted the challenge of inconsistent terminology across studies. Translating Chapter 3's findings into accessible language, such as describing epigenetics as a dimmer switch, directly addresses this gap by creating consistent, shared terms that counsellors can use. This approach equips practitioners with examples that make complex mechanisms understandable and affirming.

The accompanying client handout, Understanding the Biology of Gender Diversity (see Appendix D), demonstrates this translation by presenting key findings in clear language suitable for discussion with clients and families. Explaining that the brain and body develop on partially distinct schedules helps normalize incongruence between physical characteristics and identity (Bao & Swaab, 2011; Swaab & Garcia-Falgueras, 2009). Describing signalling molecules, environmental agents, microglia, epigenetics, and genes as dynamic systems rather than static determinants allows counsellors to convey that variation is expected in biological development (Okashita & Tachibana, 2021; Solleiro-Villavicencio et al., 2020; VanRyzin et al., 2020). Highlighting evidence for the brain as a mosaic of features commonly seen across individuals supports a spectrum-based understanding of identity rather than a binary one (Bobotis et al., 2023; Nelson et al., 2019; VanRyzin et al., 2020).

Psychoeducation grounded in this framework can reduce internalized stigma and improve family acceptance, outcomes consistently linked with better mental health trajectories for gender diverse clients (Bowers & Whitley, 2020; Spencer et al., 2021). Presenting biological complexity as a foundation for diversity enables counsellors to promote acceptance without reducing identity to a single causal explanation. Instead, it positions identity as the product of multiple interacting influences that collectively affirm human variation which includes biology, sociocultural factors, and lived experience (MacKinnon et al., 2025; Mazzuca et al., 2024; Spencer et al., 2021).

Advocacy and Interdisciplinary Work

A biologically informed framework also strengthens advocacy within healthcare, education, and policy settings. Chapter 3's demonstration of the wide range of interacting biological mechanisms that shape neurodevelopment provides counsellors with empirically grounded responses to misinformation that portrays gender diversity as a social trend, a binary system, a disorder, or as something solely socially constructed (MacKinnon et al., 2025). Grounding advocacy in evidence demonstrates the scientific legitimacy of diversity while supporting ethical obligations to use psychological and biological research responsibly (CPA, 2017). This approach fulfills the principle of responsible caring, which calls on psychologists to apply their expertise for the benefit of society and to guard against the distortion of scientific findings (CPA, 2017). Advocacy rooted in this balanced understanding also contributes to public trust in psychology and strengthens interdisciplinary collaboration across mental health and medical systems (CPA, 2017).

Contextual Factors Influencing Application of Current Research

Ethical and Legislative Factors

Chapter 2 emphasized the need to guard against ideological misuse of biological findings, a concern that becomes especially relevant amid restrictive legislation. Because this research situates the biology of gender diversity within the context of psychosocial and sociocultural interaction, it must also contend with the shifting legal terrain that shapes how those interactions are lived, recognized, and sometimes constrained. The protective legal framework includes the Alberta Human Rights Act, which prohibits discrimination based on gender, gender identity and gender expression (Government of Alberta, 2024a).

Yet a concurrent wave of restrictive statutes introduces targeted barriers for gender diverse individuals. The Health Statutes Amendment Act, 2024 limits access to gender affirming health care for minors (Government of Alberta, 2024b). The Education Amendment Act, 2024 requires parental opt in for instruction addressing gender identity and sexual orientation, and mandates parental notification regarding student name and pronoun changes (Government of Alberta, 2024c). The Fairness and Safety in Sport Act restricts participation in female-designated divisions to individuals assigned female at birth, thereby excluding many transgender and nonbinary participants (Government of Alberta, 2024d).

This structural contrast illustrates a widening legal tension where one branch of law affirms broad protections for gender identity and expression, while another imposes selective exclusions that constrain how gender diversity may be lived and supported. Importantly, this dynamic is not isolated to Alberta. Comparable legislative efforts, particularly those restricting access to gender-affirming care, educational inclusion, and participation in sport, are emerging across Canada and internationally (Government of Saskatchewan, 2023; Trans Legislation Tracker, 2025).

These legislative tensions highlight the importance of grounding biological research in ethical practice, ensuring that empirical findings inform care rather than justify restriction. As emphasized in Chapter 3, biological variation is multifactorial and referencing these mechanisms helps counter policy narratives that seek single causes of gender diversity (Bakker, 2022; Solleiro-Villavicencio et al., 2020; VanRyzin et al., 2020). By articulating how molecular, hormonal and environmental factors interface with sociocultural systems and lived experience, this project seeks to counter reductionist narratives that policy debates often weaponize to justify restriction (Abreu et al., 2022; MacKinnon et al., 2025). Counsellors must remain aware of these evolving laws to navigate confidentiality, affirming practices, and informed consent (CAP, 2023; CPA, 2017). Grounding these biological insights within counselling ethics specifically the principles of respect for dignity, responsible caring, and integrity in relationships (CPA, 2017) enables a reframing of gender diversity as a natural outcome of human neurodevelopment rather than a deviation requiring correction or control.

Real World Constraints on Disseminating Biological Information

Disseminating biological and neurodevelopmental information is shaped by practical, cultural, and systemic constraints that affect how this knowledge reaches clients, families, schools, and communities. Restrictive policy environments can amplify parental resistance to gender-affirming frameworks, particularly where legal limitations intersect with existing misconceptions (Government of Alberta, 2024b). As Grossman et al. (2021) note, parental reactions are often shaped by culturally embedded beliefs about gender, which can lead families or institutions to reject or dismiss accurate scientific explanations of gender identity.

Delivering biologically informed, gender-affirming care becomes more complex when clients hold multiple intersectional identities, because each layer of identity shapes how

biological information is accessed, interpreted, and trusted (Mendoza et al., 2020; Mezzatesta-Gava et al., 2025; Renner et al., 2021). For BIPOC clients, historical misuse of biological narratives in medicine can lead to justified skepticism toward scientific explanations of gender, requiring clinicians to present biological information with cultural humility and attention to medical mistrust (Mendoza et al., 2020).

Rural clients face a different barrier: limited availability of gender-literate clinicians reduces the accuracy and nuance with which biological mechanisms are explained, often requiring clients to navigate fragmented service pathways, rely on outdated or non-evidence-based information sources, or travel long distances to obtain affirming care (Renner et al., 2021). Neurodivergent clients encounter an additional challenge, as clinicians frequently lack awareness of autistic communication styles, sensory processing differences, and information processing needs, which can limit the clarity, accessibility, and applicability of biological explanations in practice (Mezzatesta-Gava et al., 2025). Youth add another dimension, as minors frequently navigate parental gatekeeping and cultural expectations that determine whether biological explanations are permitted or supported in their care environment (Grossman et al., 2021; Mendoza et al., 2020).

Another constraint concerns the limitations of the scientific literature itself. As outlined in chapter 2, most biological studies rely on Western, cisgender samples, which restricts the generalizability of these findings for clients whose identities intersect with race, neurodivergence, or non-Western cultural frameworks. These methodological gaps shape how confidently biological concepts can be applied across diverse client populations and require counsellors to acknowledge both what is known and the limits of generalization (CPA, 2017).

These intersecting factors compound, meaning that even accurate biological information may be constrained by structural mistrust, geographic inequities, neurodivergent communication needs, or cultural context (Mezzatesta-Gava et al., 2025). Consequently, biologically affirming care must account for how cultural, regional, cognitive, and age-related factors jointly shape the reception and applicability of gender related neurodevelopmental knowledge (Spencer et al., 2021).

In clinical settings, these dynamics can further restrict how practitioners share biological information. When parents control therapeutic content, require disclosure of session discussions, or veto affirming conversations, biological explanations may be limited even within the clinical encounter (Grossman et al., 2021). When restrictive legislation and entrenched misconceptions intersect, the scientific foundations of gender affirming care, including biological explanations, become especially vulnerable to dismissal (Grossman et al., 2021; Bowers & Whitley, 2020). Dissemination is also affected by system-level constraints. Provider training and caseload demands can restrict opportunities to translate complex biological findings into accessible psychoeducation (Valentine & Shipherd, 2018). Cultural frameworks may further shape receptivity (Mazzuca et al., 2024).

Access to counsellors who specialize in gender affirming care is shaped by provincial public funding structures, which determine which services are covered, how clients enter care pathways, and whether they receive sustained support from clinicians trained in gender diversity. These funding differences also influence the future dissemination of neurodevelopmentally informed psychoeducation, which requires adequate clinical time, continuity, and access to affirming providers.

In Alberta, the Health Statutes Amendment Act, 2024 restricts minors' access and introduces approval requirements that reduce opportunities for ongoing engagement with gender affirming clinicians (Government of Alberta, 2024a). Alberta Health Services offers limited publicly funded mental-health support through specialist clinics, where wait times often reach 12 to 24 months, and provides only short-term psychiatric care, while general low-cost counselling programs remain outside gender affirming pathways (Alberta Health Services, n.d.-a, n.d.-b; Government of Alberta, n.d.; Government of Alberta, 2024b).

In contrast, British Columbia publicly funds a coordinated, province-wide model in which psychosocial support, navigation, care coordination, and referral pathways to affirming counsellors are embedded within routine primary care through Trans Care BC's guidelines and infrastructure, thereby increasing access to gender-affirming mental health providers (Trans Care BC, 2024). When combined, these differences show that provincial public funding structures shape whether clients can reliably access counsellors trained in gender affirming practice and whether the foundational conditions for future neurodevelopmental affirmation, including adequate time, continuity, and informed providers, ever become available within the healthcare system.

Together, these constraints show that applying and disseminating biological knowledge is not merely a scientific task. It requires navigating legal restrictions, cultural expectations, institutional policies, provider capacity, and regional inequities to ensure that accurate information can be ethically and effectively integrated into practice.

Challenges in Translating the Research

Applying biological knowledge to practice requires navigating several challenges. As Chapter 2 emphasized, the fragmentation of biological research complicates synthesis, and

additional issues appear when clinicians attempt to translate Chapter 3's molecular details into therapeutic conversations. Much of the existing literature focuses on isolated mechanisms rather than integrated developmental models, which limits how easily these findings can be incorporated into clinical work. Moreover, public discussion of the biological conclusions often becomes politicized, leading to misinterpretations that imply preventability or pathology (Abreu et al., 2022; Restar et al., 2024). Practitioners must anticipate these distortions and emphasize that biological research underscores developmental diversity and plasticity rather than implying that gender identity or expression is fixed or predetermined by single factors such as hormones or chromosomal biology (MacKinnon, 2018; VanRyzin et al., 2020).

Molecular mechanisms, including hormone production, receptor polymorphisms, signalling molecules, microglial and environmental interactions, and epigenetic modulation, all work together to shape diverse developmental outcomes (Okashita & Tachibana, 2021; Solleiro-Villavicencio et al., 2020; VanRyzin et al., 2020). Communicating these nuances supports the ethical principle of integrity in relationships III.2 and ensures that biological information affirms variability rather than pathologizing difference (CPA, 2017).

Another persistent challenge involves translating dense scientific language into terms that are accurate yet accessible for clients, families, and practitioners from nonbiological disciplines, as oversimplification risks distortion, while excessive technicality can limit understanding. Finally, limited representation in existing research continues to constrain the applicability of findings, as gender-diverse and intersectional participants remain underrepresented in most biological studies. Counsellors must therefore integrate emerging evidence carefully, maintaining awareness of both its strengths and its current boundaries to ensure compliance with

straightforwardness in integrity in relationships (CPA, 2017). Ongoing studies incorporating gender diverse and cross-cultural samples will be essential to refine and validate these models.

Recommendations for Clinical Practice

Integrating biological synthesis into counselling practice enhances several dimensions of gender affirming care. The Gender Affirmative Lifespan Approach (Spencer et al., 2021) remains a central framework, and embedding biological understanding within it deepens its scope and applicability. Conceptualizing gender diversity as a natural biological variation reinforces affirmation at the level of science while grounding practice in evidence rather than assumption (Spencer et al., 2021; MacKinnon, 2018). When counsellors understand neurodevelopmental processes, they strengthen their gender literacy and can communicate biological information in ways that validate client experience while dispelling misconceptions (Spencer et al., 2021).

Biological variability also reflects the intersectional complexity of human identity, reminding practitioners that gendered development arises through multiple interacting systems rather than a single binary pathway (Spencer et al., 2021; Mazzuca et al., 2024). Chapter 3's findings collectively demonstrate how multiple biological systems generate a spectrum of possible developmental outcomes (VanRyzin et al., 2020). Referencing these mechanisms in practice reinforces affirmation grounded in empirical evidence. Within this integrated model, affirmation is both scientific and ethical, founded on respect for human dignity and diversity, informed by converging evidence across molecular, developmental, and psychological domains (CPA, 2017).

Psychoeducational and therapeutic applications can extend these principles beyond individual sessions and into broader systems of support. Parent and caregiver groups can use the client handout as a foundation for dialogue about how biological mechanisms support rather than

contradict diversity. Youth programs may integrate brief explanations of neurodevelopmental processes into identity-affirmation activities so that participants can recognize their experiences as part of the normal range of human variation. Interdisciplinary workshops for educators and healthcare professionals can also build biological literacy, reduce stigma and improve collaboration across networks of care (MacKinnon et al., 2025).

Embedding biological literacy within training and supervision also enhances professional competence. Graduate coursework can pair neurodevelopmental science with counselling theory to illustrate how biology and psychology interact in shaping identity. Supervisors can encourage trainees to reflect on how their own assumptions intersect with emerging scientific evidence, using these discussions to refine ethical and clinical judgment. Practicum seminars can use case studies to model how biological information can be integrated into psychoeducation and client formulation. These strategies align with CAP (2023) requirements for maintaining competence and reflect counselling psychology's broader commitment to social justice and evidence-based care. Chapter 2's integrative method, which centered on mechanisms with clear developmental impact, supports embedding these findings directly into GALA and into the following proposed Neurodevelopmental Approach to Gender Affirmation (NAGA) formulations.

Proposed Theoretical Extension Neurodevelopmental Approach to Gender Affirmation

To operationalize this biological synthesis in practice, the following applied framework called NAGA is proposed. This approach uses the biological processes described in Chapter 3, translated into the accessible language developed in Appendix D, to inform three core tasks of affirming counselling practice, exploration, validation, and integration. NAGA provides language for connecting developmental diversity in biology with the broader understanding that human variation strengthens relationships, communities, and systems (Kamalumpundi et al., 2024). It

functions as a bridge between molecular science and lived experience, ensuring that biological knowledge enhances rather than overshadows the personal, relational, and cultural dimensions of identity. In this way, NAGA positions biology as context rather than cause, supporting affirming care with scientific precision while respecting clients' self-defined meanings.

Within NAGA, exploration invites clients to consider how learning about neurodevelopmental diversity may shape their understanding of identity and embodiment. This process remains client-led; some individuals may find biological knowledge stabilizing or affirming, others may weave it into a broader constellation of identity narratives, and others may find it minimally relevant. Exploration requires flexibility, especially for clients whose cultural contexts, developmental stage, or cognitive processing styles influence how biological narratives are interpreted.

Validation situates lived experience within what is known about human development. When counsellors present biology as evidence of natural variation, identity becomes framed within empirical knowledge rather than outside of it. This helps counter pathologizing narratives and reinforces the view that diversity is expected within human neurodevelopment (MacKinnon, 2018). Validation also adapts to intersectional needs, for example, as discussed earlier, offering biological information with cultural humility for BIPOC clients who may carry justified medical mistrust, or presenting it in sensory and processing accessible formats for neurodivergent clients (Mendoza et al., 2020; Mezzatesta-Gava et al., 2025; Spencer et al., 2021).

Integration carries the process outward by connecting individual understanding to broader relational, cultural, and community systems. Diversity at the molecular and cellular levels mirrors diversity across families, cultures, and societies (Kamalumpundi et al., 2024; Mazzuca et al., 2024). This micro-macro parallel reinforces the principle that variation is a defining feature

of living systems and a foundation for adaptability, resilience, and creativity. Integration also acknowledges the practical realities that shape how biological knowledge can be applied, including parental gatekeeping, regional inequities in provider expertise, cultural frameworks that conceptualize gender differently, and the developmental needs of youth (Mazzuca et al., 2024; Renner et al., 2021; Restar et al., 2024; Valentine & Shipherd, 2018).

Together, these three tasks ensure that NAGA remains grounded in the biological evidence reviewed in Chapter 3 while also responsive to the intersecting contexts that shape how client's access, interpret, and use biological information. NAGA therefore offers a flexible, developmentally informed framework that supports exploration, affirms lived experience, and extends biological understanding into relational and cultural domains where identity is lived and supported.

Summary and Implications

The findings of Chapter 4 demonstrate that applying biological research to counselling practice requires attention not only to the developmental mechanisms themselves but also to the broader systems that shape their implementation and dissemination, including legislation, parental gatekeeping, cultural frameworks, provider training, and regional inequities (Mazzuca et al., 2024; Renner et al., 2021; Restar et al., 2024; Valentine & Shipherd, 2018). Bringing neurodevelopmental evidence into practice strengthens exploration, validation, and affirmation by framing gender diversity as an expected outcome of human development rather than a deviation (MacKinnon et al., 2025; Spencer et al., 2021).

When dissemination of affirming biological information is constrained by misconceptions, restrictive laws, systemic barriers, or limited access to affirming providers the ability of biological knowledge to reduce stigma and counter misinformation becomes harder to

realize (Grossman et al., 2021; Restar et al., 2024). By contrast, when these constraints are acknowledged and navigated ethically, neurodevelopmental research can meaningfully support advocacy and deepen exploration and validation in practice (Spencer et al., 2021). Taken together, these applications and constraints illustrate that translating biological knowledge into counselling practice requires both scientific clarity and contextual awareness.

Chapter 4 has shown how neurodevelopmental evidence can support exploration, validation, and gender affirming care, while also demonstrating how legal, systemic, and cultural forces shape what is possible in practice (Bakker, 2022; Restar et al., 2024; Spencer et al., 2021). These insights set the stage for Chapter 5, which synthesizes the project's contributions, addresses methodological limitations, and outlines future directions by situating the findings within the broader landscape of counselling psychology and illustrating how an integrated biological framework can inform ethical practice, research development, and the ongoing expansion of affirming care.

Chapter 5: Recommendations and Conclusion

Summary of the Study

This capstone synthesized current biological and neurodevelopmental research to clarify how genetics, hormones, epigenetics, microglia, and environmental factors interact to shape gender diversity. Guided by neurodevelopmental theory, the study demonstrated that gender identity is not a social anomaly but an expected outcome of human neurodevelopment. The problem addressed was the absence of a unified, counselling-relevant synthesis of biological evidence explaining gender diversity. Without this synthesis counselling psychology risks reinforcing outdated binary models that undermine affirming care (MacKinnon et al., 2025; Spencer et al., 2021).

Across the study, evidence converged on three key points. Brain and body differentiation follow separate but overlapping critical periods (Bakker, 2022; Swaab & Garcia-Falgueras, 2009), allowing divergence between anatomical and neural outcomes. Molecular and cellular systems (McCarthy et al., 2018), hormones (Nelson et al., 2019), receptors (Fernández et al., 2018), neurotransmitters (Martinez Ramirez et al., 2023), endocannabinoids (Blanton et al., 2021), genetics (Okashita & Tachibana, 2021), epigenetics (Kundakovic & Tickerhoof, 2024), and microglia (VanRyzin et al., 2020), act in concert to produce wide variation within typical human development. Environmental exposures can modulate these pathways but do not cause gender diversity, instead, they illustrate how context and biology continually interact (Santoro et al., 2021; VanRyzin et al., 2020).

Together, these findings establish that the spectrum of gender diversity is biologically grounded, developmentally natural, and ethically significant for counselling psychology in its roles of affirmation, validation, exploration, and countering misinformation.

The Take Home Message

Gender diversity is a natural expression of human neurodevelopment. It arises through the same molecular and cellular processes that generate every other form of variation in living systems (VanRyzin et al., 2020). Differences that society has learned to fear or pathologize are, in scientific fact, evidence of the adaptive range that sustains our species (Bobotis et al., 2023; Kamalumpundi et al., 2024). For communities and institutions, this means that the biological uniqueness of gender diverse individuals should inspire curiosity, respect, and celebration, not correction. For counsellors, it means taking an active role in translating and disseminating this knowledge, using it to support clients in exploration, validation, and advocacy (Spencer et al., 2021). By grounding affirmation in science, counsellors help shift social narratives from fear to understanding and from correction to connection, reinforcing that diversity is both natural and beneficial for the prosperity and adaptability of our shared communities (Kamalumpundi et al., 2024; Spencer et al., 2021).

Recommendations for Future Research

Building on the integrative framework established here, future research can extend knowledge in three interconnected directions. The first is to map neurodevelopment in relation to gender across the entire lifespan. Additional sensitive and critical windows such as mini puberty and puberty represent secondary periods of heightened neuroendocrine plasticity and continued gendered neurodifferentiation (Bakker, 2022; VanRyzin et al., 2020). These stages offer valuable opportunities to observe how hormonal surges recalibrate microglial activity, receptor sensitivity, and circuit organization in relation to identity formation. Longitudinal imaging and molecular studies could clarify how early neurodevelopmental pathways interact with later critical periods

of differentiation and lived perceptual, behavioral, and relational experiences, offering a more complete picture of developmental continuity (Bakker, 2022; VanRyzin et al., 2020).

The second recommendation is to construct a comprehensive Neuro Functional Atlas of human neurodevelopment. Using this study's template, future research could catalogue the full range of biological components involved in brain formation, including hormones, neurotransmitters, endocannabinoids, transcription factors, receptors, glia, precursor cells, transport proteins, genes, and epigenetic regulators, among other contributors to neurodevelopment. Such a map would allow cross-disciplinary modelling of how multiple systems codetermine identity formation and could identify new therapeutic or educational applications.

A third direction is to expand research on environmental modulation of neurodevelopment. This includes developing a comprehensive map of environmental factors that influence neurodevelopment, which could naturally complement the molecular Neuro Functional Atlas described above. Such research would examine how elements such as diet, pharmaceuticals, regional environmental exposures, and lifestyle factors shape molecular signalling during development. Comparative studies across ecological and cultural contexts could reveal regional variability in developmental inputs and clarify how local environments interact with biological systems. Across all these future recommendations, participant diversity remains essential. Future studies must intentionally include gender diverse, culturally diverse, and regionally varied populations. Doing so will increase generalizability, address the current cisgender and Western sample bias, and uphold the Tri-Council principle that no group should be unjustly excluded from the benefits of research (Canadian Institutes of Health Research et al., 2022; MacKinnon et al., 2025; Spencer et al., 2021).

Limitations and Methodological Considerations

Reliance on prior integrative reviews highlights how building upon existing science is inevitably influenced by the interpretation and synthesis of other researchers, which was mitigated through comparative reading, attention to areas of convergence and contradiction among reviews, and evaluation of the evidence base supporting each synthesis. The fragmentation of disciplinary islands highlights the ongoing need for translational frameworks that bridge molecular and applied knowledge. The persistence of binary and Western sampling patterns exposes structural biases that mirror broader societal inequities, reinforcing the ethical imperative for inclusive and globally representative research (Canadian Institutes of Health Research et al., 2022; MacKinnon et al., 2025).

Terminological inconsistency across studies illustrates how language shapes scientific understanding and how binary assumptions have shaped the knowledge system of science itself. This means that even before data are collected, worldviews are already embedded in what counts as legitimate evidence. The reliance on animal models (Bordt et al., 2020) and older foundational work (Swaab & Garcia-Falgueras, 2009) reveals both the constraints and continuity of biological inquiry. Collectively, these factors demonstrate that the limitations of this research extend beyond technical issues of method to include the underlying assumptions that shape how knowledge about gendered neurodevelopment is defined, interpreted, ethically navigated, and applied.

Addressing these limitations requires methodological strategies that prioritize inclusivity, transparency, and conceptual precision (MacKinnon, 2018). Future integrative reviews could strengthen validity by expanding database coverage and comparing overlapping syntheses to identify convergence and divergence. Sustaining cross-disciplinary integration among cellular,

neural, and environmental sciences, while extending this approach to include disciplines such as neuroimaging, behavioral research, and systems neuroscience would help ensure that future syntheses maintain coherence across biological and experiential levels of analysis.

Greater consistency could also be achieved if future primary researchers and review authors collaborated to align terminology for concepts currently described with multiple overlapping terms. Even partial consensus on key definitions would reduce discrepancies in interpretation arising from terminology inconsistency, improving comparability across studies. Collectively, these measures would enhance the reliability and representativeness of future research.

Beyond shared terminology, a more coordinated model of inquiry could be established in which primary investigators and synthesis authors intentionally align research trajectories to reduce redundancy and strengthen complementarity (Barres, 2018). Through structured dialogue on emerging findings, methodological gaps, and priority questions, each group could identify which lines of investigation are most likely to yield new insight or social benefit. A dedicated interdisciplinary consortium could situate these collaborations within a broader meta framework, conceptually parallel to initiatives such as the Human Cell Atlas and the BRAIN Initiative, which exemplify large scale cooperative mapping of cells and circuits, yet extended to encompass the neural structures and processes that underpin perceptual, cognitive, and affective outcomes such as intelligence, creativity, empathy, and identity.

Such a Neuro Functional Atlas would link molecular, cellular, and circuit level organization to complex behavioral and experiential dimensions, providing a coherent scaffold through which both primary studies and integrative syntheses could contribute to a shared and continuously evolving body of knowledge.

Ethical Reflection on the Dual Use Potential of Biological Knowledge

As discussed earlier regarding methodological integrity, preventing the weaponization of biological findings is essential to ethical research (Restar et al., 2024). Yet beyond methodological safeguards lies a broader tension as the same developmental mapping that clarifies the biological foundations of gender diversity can also be applied to control or erase that variation (Restar et al., 2024). Any framework capable of elucidating neurodevelopmental diversity carries a parallel risk of being misused to constrain it.

Understanding this dual-use potential reinforces the need to ground biological inquiry within ethical principles that prioritize dignity, autonomy, and diversity over prediction or correction (CAP, 2023; CPA, 2017; Canadian Institutes of Health Research et al., 2022; Restar et al., 2024). It also calls for proactive dialogue between researchers, clinicians, and policymakers to ensure that the expanding biological understanding of gender serves affirming rather than regulatory ends (Canadian Institutes of Health Research et al., 2022). In this sense, ethical care begins long before findings reach the public; it is embedded in how questions are framed, which comparisons are made, and whose perspectives are included in defining what counts as knowledge.

Personal and Professional Reflection

This project reshaped my scientific understanding, my counselling conceptualization, and my ethical stance toward biological knowledge, clarifying how each domain mutually informs the others. Through the iterative process of synthesis across fragmented disciplines, I transitioned from focusing on isolated biological correlates to recognizing the importance of multi-layered developmental systems. This shift reshaped not only how I understood gendered neurodevelopment but how I approach conceptualization more broadly. My learning expanded

from cataloguing biological correlates to understanding them as co-created processes that gain significance through their interactions. This informs counselling work as well, where cross-system patterns uncover meaning beyond their individual parts.

As the project developed, I also found that microglia provided a connection point through which my previously scattered findings could be organized. Once I honed in on microglia and their potential role in gendered neurodevelopment, it became possible to trace a chronological story of how diverse factors shape the developmental blueprints that microglia act upon in gendered ways. Sharing this emerging narrative with others highlighted an unexpected but important insight, that narrative coherence substantially increases comprehension and impact. The developmental story resonated more strongly than isolated correlational evidence, and the individuals I discussed it with consistently found the narrative structure more digestible, meaningful, and profound. This recognition will inform my clinical practice, underscoring the value of using developmental narratives to support client understanding and identity exploration.

Throughout this work, the recurrent need to evaluate the ethical dimensions of what counts as scientific knowledge, as well as the moral implications of how that knowledge may be applied, strengthened my appreciation for how knowledge systems shape practice. Engaging with these considerations made clear that scientific understanding and ethical responsibility are inseparable in counselling psychology, and that biological insights must always be situated within frameworks that uphold dignity, autonomy, and diversity.

This learning clarified that ethical care begins upstream, within the very structures that organize what counts as knowledge, then shaped how I imagine larger integrative models, such as a Neuro Functional Atlas. Recognizing this interdependence deepened my commitment to translating complex findings into forms that are usable within clinical settings, and it further

clarified the role counsellors play in bridging science and human experience in ways that make knowledge accessible, meaningful, and affirming.

As the literature analysis evolved, my perspective shifted from interpreter to collaborator, envisioning how primary researchers, synthesizers, and practitioners might cocreate knowledge systems that reflect the same inclusivity and respect sought in therapeutic relationships. This process also reinforced that part of the foundation of both research and counselling lies in humility, the capacity to remain open, responsive, and aware of one's own limits when engaging with complexity.

Humility, in this sense, represents more than personal modesty; it is a knowledge-based, ethical, and integrative stance. Knowledge based humility recognizes that all understanding is partial and keeps inquiry open to new perspectives and evidence. Ethical humility affirms the dignity and complexity of persons and systems, guarding against reductionism or overconfidence in interpretation. Integrative humility serves as the connective principle that allows multiple levels of knowledge, molecular, neural, environmental, and experiential, to coexist without hierarchy. For me as a developing counselling psychologist, this recognition links the ethics of research design and collaboration directly to the ethics of practice as both call for transparency and a sustained commitment to human diversity as a source of insight rather than difference to be resolved (CAP, 2023; CPA, 2017).

Implications for Clinical Practice and Professional Development

The insights synthesized in this capstone reaffirm several principles that can guide clinical practice and professional development moving forward. For assessment, an understanding of gendered neurodevelopment encourages clinicians to contextualize identity exploration within developmental, neurobiological, and environmental systems rather than

within deficit-based or binary diagnostic frames. In psychoeducation, the developmental narrative emerging from this synthesis offers a foundation for scripts that translate complex biological information into accessible, client centered explanations that validate diversity without pathologizing it.

The findings also highlight messaging principles that emphasize coherence, clarity, and respect, particularly when addressing misconceptions about the origins of gender diversity. This includes normalizing variation, foregrounding developmental pathways, and inviting curiosity rather than correction. In terms of advocacy, the biological evidence consolidates a rationale for supporting affirming policies, challenging misinformation, and promoting equitable access to gender affirming care.

Finally, the interdisciplinary scope of this work has implications for practitioner training. Counsellors benefit from literacy across biological, developmental, and sociocultural domains, as well as the ethical skills required to translate scientific knowledge responsibly. Incorporating neurodevelopmental literacy, narrative framing, and upstream ethical reflection into training programs can strengthen the profession's capacity to provide informed, inclusive, and scientifically grounded care.

Conclusion

Returning to the problem that first shaped this inquiry, the absence of a unified biologically grounded framework within counselling psychology, integration requires not only scientific synthesis but also ethical humility. The knowledge traced through this capstone demonstrates that understanding gender diversity demands a stance that values precision without presumption and connection without reduction. Grounding affirmation in both biology and ethics ensures that the field evolves toward inclusivity without losing rigor and toward complexity

without losing care (Canadian Institutes of Health Research et al., 2022). In this way, the principles that guide ethical practice also guide ethical knowledge where respect, openness, and humility become how counselling psychology continues to expand its understanding of human diversity. Taken as a whole, this project demonstrates how integrated biological knowledge can strengthen affirming counselling practice, inform future research directions, and support advocacy that upholds the dignity and diversity of gender diverse communities.

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Appendix A

Boolean Search Strategies

The following Boolean search strategies were used across databases to identify peer reviewed literature relevant to biological and neurodevelopmental processes underlying gender diversity. Each configuration was tested iteratively to evaluate conceptual coverage, relevance, and term interactions. The final search strings are presented below for transparency and reproducibility.

PubMed

1.

("gender diversity"[Title/Abstract] OR "gender spectrum"[Title/Abstract] OR "gender identity"[Title/Abstract] OR transgender[Title/Abstract] OR "nonbinary"[Title/Abstract] OR "differences in sexual development"[Title/Abstract])
AND
(neurodevelopment[Title/Abstract] OR "critical periods"[Title/Abstract] OR "neurological differentiation"[Title/Abstract] OR "sexual differentiation"[Title/Abstract] OR "neurological sex differences"[Title/Abstract] OR "sex differences"[Title/Abstract] OR genetics[Title/Abstract] OR epigenetics[Title/Abstract] OR receptors[Title/Abstract] OR hormones[Title/Abstract] OR estrogen[Title/Abstract] OR androgen[Title/Abstract] OR endocannabinoids[Title/Abstract] OR fetus[Title/Abstract] OR "fetal development"[Title/Abstract] OR prenatal[Title/Abstract] OR "endocrine disruptors"[Title/Abstract] OR "environmental teratogens"[Title/Abstract] OR "glial cells"[Title/Abstract] OR astrocytes[Title/Abstract] OR microglia[Title/Abstract])
AND
("Journal Article"[pt] OR "Review"[pt])

2.

(fetus[Title/Abstract] OR "fetal development"[Title/Abstract] OR prenatal[Title/Abstract])
AND
(neurodevelopment[Title/Abstract] OR "critical periods"[Title/Abstract] OR "neurological differentiation"[Title/Abstract] OR "sexual differentiation"[Title/Abstract] OR "neurological sex differences"[Title/Abstract] OR "sex differences"[Title/Abstract])
AND
(("gender diversity"[Title/Abstract] OR "gender spectrum"[Title/Abstract] OR "gender identity"[Title/Abstract] OR transgender[Title/Abstract] OR "nonbinary"[Title/Abstract] OR "differences in sexual development"[Title/Abstract])
OR (genetics[Title/Abstract] OR epigenetics[Title/Abstract] OR receptors[Title/Abstract] OR hormones[Title/Abstract] OR estrogen[Title/Abstract] OR androgen[Title/Abstract] OR endocannabinoids[Title/Abstract])
OR ("endocrine disruptors"[Title/Abstract] OR "environmental teratogens"[Title/Abstract])
OR ("glial cells"[Title/Abstract] OR astrocytes[Title/Abstract] OR microglia[Title/Abstract]))
AND
("Journal Article"[pt] OR "Review"[pt])

3.
(fetus[Title/Abstract] OR "fetal development"[Title/Abstract] OR prenatal[Title/Abstract])
AND
(("gender diversity"[Title/Abstract] OR "gender spectrum"[Title/Abstract] OR "gender identity"[Title/Abstract] OR transgender[Title/Abstract] OR "nonbinary"[Title/Abstract] OR "differences in sexual development"[Title/Abstract])
OR (neurodevelopment[Title/Abstract] OR "critical periods"[Title/Abstract] OR "neurological differentiation"[Title/Abstract] OR "sexual differentiation"[Title/Abstract] OR "neurological sex differences"[Title/Abstract] OR "sex differences"[Title/Abstract])
OR (genetics[Title/Abstract] OR epigenetics[Title/Abstract] OR receptors[Title/Abstract] OR hormones[Title/Abstract] OR estrogen[Title/Abstract] OR androgen[Title/Abstract] OR endocannabinoids[Title/Abstract])
OR ("endocrine disruptors"[Title/Abstract] OR "environmental teratogens"[Title/Abstract])
OR ("glial cells"[Title/Abstract] OR astrocytes[Title/Abstract] OR microglia[Title/Abstract]))
AND
("Journal Article"[pt] OR "Review"[pt])

4.
(neurodevelopment[Title/Abstract] OR "critical periods"[Title/Abstract] OR "neurological differentiation"[Title/Abstract] OR "sexual differentiation"[Title/Abstract] OR "neurological sex differences"[Title/Abstract] OR "sex differences"[Title/Abstract])
AND
(("gender diversity"[Title/Abstract] OR "gender spectrum"[Title/Abstract] OR "gender identity"[Title/Abstract] OR transgender[Title/Abstract] OR "nonbinary"[Title/Abstract] OR "differences in sexual development"[Title/Abstract])
OR (genetics[Title/Abstract] OR epigenetics[Title/Abstract] OR receptors[Title/Abstract] OR hormones[Title/Abstract] OR estrogen[Title/Abstract] OR androgen[Title/Abstract] OR endocannabinoids[Title/Abstract])
OR (fetus[Title/Abstract] OR "fetal development"[Title/Abstract] OR prenatal[Title/Abstract])
OR ("endocrine disruptors"[Title/Abstract] OR "environmental teratogens"[Title/Abstract])
OR ("glial cells"[Title/Abstract] OR astrocytes[Title/Abstract] OR microglia[Title/Abstract]))
AND
("Journal Article"[pt] OR "Review"[pt])

PsycINFO

1.
TI,AB(("gender diversity" OR "gender spectrum" OR "gender identity" OR transgender OR "nonbinary" OR "differences in sexual development"))
AND
TI,AB((neurodevelopment OR "critical periods" OR "neurological differentiation" OR "sexual differentiation" OR "neurological sex differences" OR "sex differences" OR genetics OR

epigenetics OR receptors OR hormones OR estrogen OR androgen OR endocannabinoids OR fetus OR "fetal development" OR prenatal OR "endocrine disruptors" OR "environmental teratogens" OR "glial cells" OR astrocytes OR microglia))

2.
TI,AB((fetus OR "fetal development" OR prenatal))
AND
TI,AB((neurodevelopment OR "critical periods" OR "neurological differentiation" OR "sexual differentiation" OR "neurological sex differences" OR "sex differences"))
AND
TI,AB((("gender diversity" OR "gender spectrum" OR "gender identity" OR transgender OR "nonbinary" OR "differences in sexual development")
OR (genetics OR epigenetics OR receptors OR hormones OR estrogen OR androgen OR endocannabinoids)
OR ("endocrine disruptors" OR "environmental teratogens")
OR ("glial cells" OR astrocytes OR microglia)))

3.
TI,AB((fetus OR "fetal development" OR prenatal))
AND
TI,AB((("gender diversity" OR "gender spectrum" OR "gender identity" OR transgender OR "nonbinary" OR "differences in sexual development")
OR (neurodevelopment OR "critical periods" OR "neurological differentiation" OR "sexual differentiation" OR "neurological sex differences" OR "sex differences")
OR (genetics OR epigenetics OR receptors OR hormones OR estrogen OR androgen OR endocannabinoids)
OR ("endocrine disruptors" OR "environmental teratogens")
OR ("glial cells" OR astrocytes OR microglia)))

4.
TI,AB((neurodevelopment OR "critical periods" OR "neurological differentiation" OR "sexual differentiation" OR "neurological sex differences" OR "sex differences"))
AND
TI,AB((("gender diversity" OR "gender spectrum" OR "gender identity" OR transgender OR "nonbinary" OR "differences in sexual development")
OR (genetics OR epigenetics OR receptors OR hormones OR estrogen OR androgen OR endocannabinoids)
OR (fetus OR "fetal development" OR prenatal)
OR ("endocrine disruptors" OR "environmental teratogens")
OR ("glial cells" OR astrocytes OR microglia)))

ScienceDirect

1.
("gender diversity" OR transgender)
AND
(neurodevelopment OR "sex differences")
AND
(hormones OR genes OR microglia)

2.
(prenatal OR "fetal development")
AND
(neurodevelopment OR "critical periods")
AND
("gender diversity" OR transgender OR "sex differences")

3.
(prenatal OR fetus)
AND
(hormones OR genes)
AND
(microglia OR astrocytes)

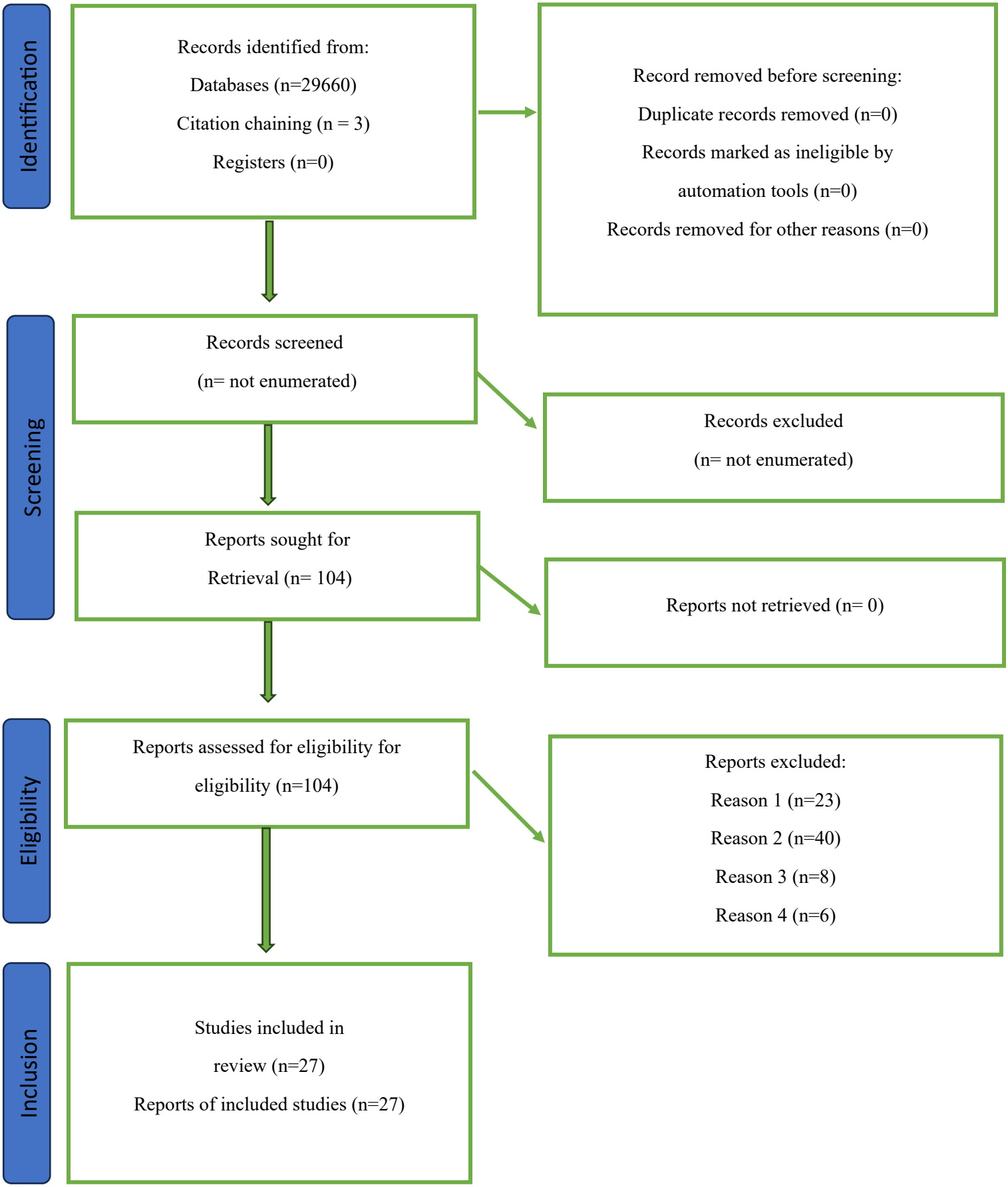
4.
(neurodevelopment OR "critical periods")
AND
("gender diversity" OR transgender OR "sex differences")
AND
(microglia OR astrocytes)

5.
(neurodevelopment AND "sexual differentiation")
AND
(hormones OR genes OR epigenetics)
AND
(prenatal OR fetus)

6.
("endocrine disruptors")
AND
(prenatal)
AND
("gender diversity" OR transgender OR neurodevelopment OR "hormones" OR "receptors" OR microglia)

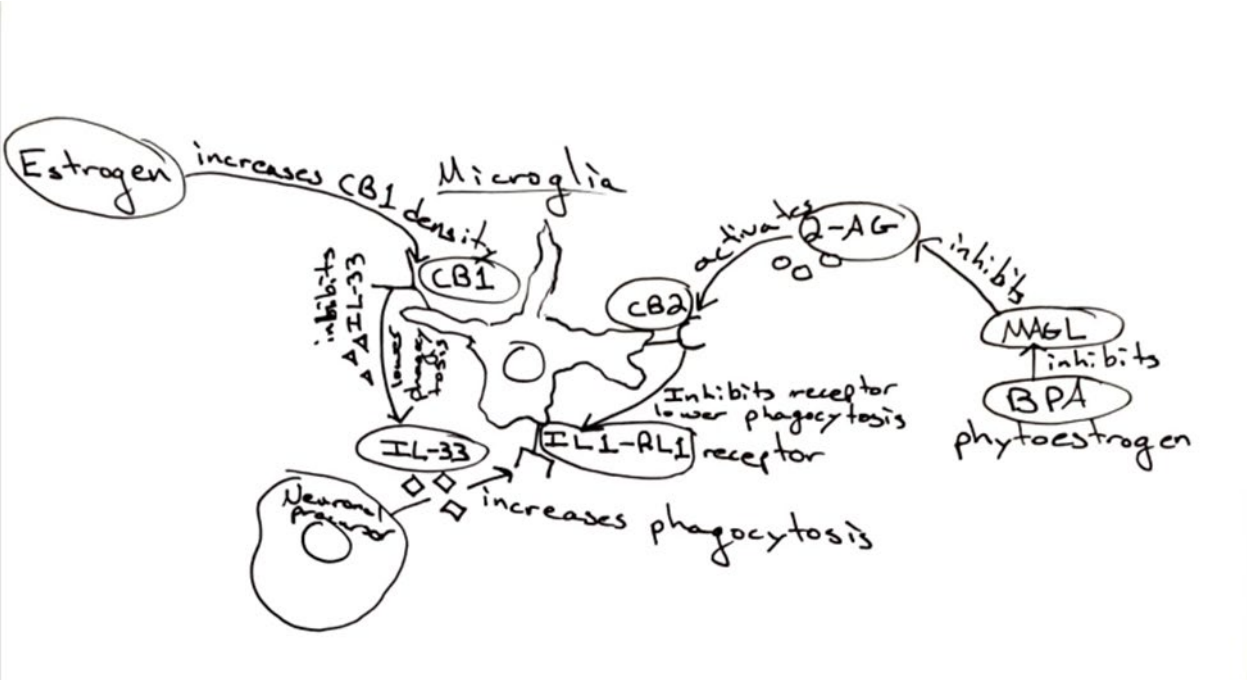
Appendix B

PRISMA Figure



Note. Reasons for full-text exclusion were as follows: Reason 1, did not address prenatal development or developmental biological mechanisms. Reason 2, did not advance step-by-step molecular or developmental account of gendered neurodevelopment. Reason 3, insufficient empirical evidence to support a coherent developmental mechanism. Reason 4, superseded by more recent evidence.

Appendix C


Estrogen
increases CB1 density
Microglia
CB1
CB2
2-AG
activates
inhibits
MAGL
inhibits
BPA
phytoestrogen
inhibits IL-33
lower phagocytosis
Inhibits receptor
lower phagocytosis
IL-33
IL1-RL1 receptor
Neuronal Axon
increases phagocytosis

Appendix D

Understanding the Biology of Gender Diversity

Why Biology Matters

Gender diversity has always been part of human experience. While culture and personal identity play important roles, research shows that biology also contributes to why people experience gender in diverse ways. Knowing this can help counter stigma and affirm that gender diversity is a natural and valid part of human variation. Biology helps explain diversity, not limit it. It shows that many different pathways can lead to healthy and authentic identities.

Brain and Body Development Happen on Different Timelines

Timeline of Development	
Weeks 6 - 12	**Body begins differentiation**
Weeks 8 - 24	**Brain critical period of differentiation**

During pregnancy, the body begins developing as male, female, or intersex around weeks 6–12. The brain, however, has its own “critical period” of development from about weeks 8–24. Because these windows overlap but don’t fully match, the brain and body can follow different developmental paths. This helps explain why someone’s gender identity may not align with the sex assigned at birth.

Factors that Shape Gender Development

Once the body and brain begin developing on their own schedules, a variety of biological factors guide how those processes unfold:

- Signalling molecules such as estrogen, testosterone, neurotransmitters, and cortisol influence brain development. It is their timing and levels during pregnancy that matter

most for how the brain develops. The interactions these molecules have with each other and with our cells influence how brain cells grow and connect.

- Epigenetics, a “dimmer switch” for our DNA, and genes affect how sensitive brain cells are to these signals. Small genetic variations in hormone receptors, for example, can shape how brains and bodies form and affect their experience of gender.
- Environmental agents can sometimes alter hormone activity. While they may influence outcomes, it is important to stress that gender diversity is not caused by “contamination.” It results from a complex interplay of many factors.
- Microglia, the brain’s “gardeners,” help sculpt our brains by adding or trimming connections and responding to the instructions they receive from signaling molecules to maintain, divide, or remove neurons. Importantly, research shows that hormones, other signalling molecules, genetics, epigenetics, and even environmental influences can all change microglial activity. These changes, in turn, influence how the brain develops in gendered ways, for example, by affecting the thickness of certain brain regions or how strongly different areas are connected to each other. These influences build on one another like layers, with each adding new possibilities for how gender can develop across the wide spectrum of human experience.

Evidence for a Spectrum

These many layers of influence make it clear that there is no single path for how gender develops. Every brain is made up of a mix of features, some more common in men, some more common in women, and some unique. This mix is evidence that gendered neurological outcomes naturally exist on a spectrum rather than in two boxes. The diversity seen in our brains and bodies mirrors the diversity found among people, perspectives, families, and cultures.

Why This Knowledge Helps

- **Affirmation**: Knowing that gender diversity is rooted in biology can counter harmful myths that it is a “choice” or “phase.”
- **Self-understanding**: Clients may feel relief in recognizing their experiences have a biological foundation.
- **Family and community support**: Families often find reassurance in scientific explanations, which can increase acceptance.
- **Reducing stigma**: Counsellors can use this knowledge to challenge misinformation and support client-centred care.
- **Diversity as strength**: Biological and human diversity both help systems adapt and thrive. Scientific studies show that diversity is not only natural but beneficial. In both nature and communities, it fosters creativity, resilience, and the ability to adapt and thrive. Systems that restrict our natural forms of identity and expression narrow the richness of our shared humanity and diminish us all.

Key Takeaway

Gender diversity is a natural outcome of how complex biological systems work together during development. Just as no two people’s fingerprints are the same, no two brains follow the same developmental path. Biology is one piece of the puzzle, interwoven with personal, social, and cultural experiences that together shape each person’s unique and valid gender identity.

A Story from the Inside: Riding Along with a Microglia

To make this more concrete, imagine a tiny guide named Mika the microglia. You can picture climbing into a very small space suit and riding along with Mika as their story unfolds.

Stage 1: Starting in the yolk sac and following the growing rivers

Mika begins life in the yolk sac, outside the early brain. Mika is created along with many other microglia. They are like gardeners in training, waiting for the signal to travel.

Around this time, the body has not yet fully begun its visible gender related development, but the early instructions that will later guide body differentiation are starting to line up. Genes that help form testes or ovaries are preparing to turn on, and hormone signals are beginning to organize. The body's project is getting ready to begin.

To reach the brain, Mika needs rivers. Those rivers are the new blood vessels that grow between the yolk sac and the tiny developing brain. A signal called estrogen helps grow these vessels by increasing something called VEGF which acts as a "grow more rivers" message.

If estrogen levels are higher at the right time, these rivers grow quickly. Mika and their microglia friends can travel sooner and arrive in the brain earlier. If estrogen levels are lower or the timing is different, the rivers grow more slowly. Mika's journey is delayed, and they arrive later.

Even small changes in timing mean that when Mika finally steps off their "boat" into a brain region, the local environment is slightly different. Some signals may be stronger or weaker. Mika has to respond to whatever they find when they arrive.

Stage 2: Entering a changing brain

By the time Mika enters the brain, they arrive early and keep spreading and multiplying as the brain develops. When the brain reaches its long critical period for gender related

development, Mika is already there and ready to begin shaping circuits as those sensitive windows open.

Everywhere Mika goes, they listen for messages:

• Hormones such as testosterone, estrogen, and cortisol

• Brain helpers such as endocannabinoids, chemical messengers that guide how active different areas should be

• Instructions from genes and their epigenetic dimmers, the system that turns certain instructions up or down

• Gentle influences from the outside world, which can subtly change how these other messages behave

These messages do not tell Mika “make this brain male” or “make this brain female.” Instead, they give detailed instructions about which neurons to multiply, which ones to protect, which to prune, and how active to be in each region.

In one brain area, strong androgen messages might ask Mika to clear away certain neurons so that circuits that support one style of behavior can grow. In another area, changes in estrogen or stress hormones might ask Mika to protect, prune, or multiply, neurons linked to emotion, memory, or how we interact with the world.

If influences from the outside world shift how hormones or messengers behave, Mika adjusts. Mika cannot see where these messages came from. They only know that the instructions have changed, so they adjust their growth, pruning, and maintenance accordingly.

Stage 3: Reading the blueprints

You can think of all these influences as blueprints that keep being updated. There is no single male or female blueprint. Each brain develops its own version, shaped by timing, signals,

and the surroundings the brain is growing in, and these variations can fall anywhere across the gender spectrum. Genetics draw the basic outline. Epigenetics adjusts how bold or faint different lines are. Hormones, stress signals, and endocannabinoids add little glowing arrows on the blueprint that show which areas need more shaping or extra care. Gentle influences from the wider world can soften or stretch the blueprint's curves, giving some areas a slightly different shape.

Mika's job is to read these living, changing blueprints and shape the brain's wiring to match them. In some people, the pattern that takes shape fits closely with the bodily sex pattern. In others, the inner pattern is different. The brain's "gendered" networks may align more with another gender, blend features, or sit somewhere in between. From Mika's point of view, all of these outcomes are simply different valid results of the same basic instructions and tools. Mika is not correcting mistakes. Mika is faithfully following the signals they receive.

Stage 4: Many journeys, many outcomes

Across the whole brain, millions of microglia like Mika repeat this process. They arrive at slightly different times, in slightly different environments, with slightly different combinations of signals. Over weeks and months, they help create a unique pattern of connections in each person. This is one reason why no two brains are exactly alike. It is also one reason why gender can emerge in many different ways, even among people with the same chromosomal pattern or similar bodies.