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Genomic Psychiatry

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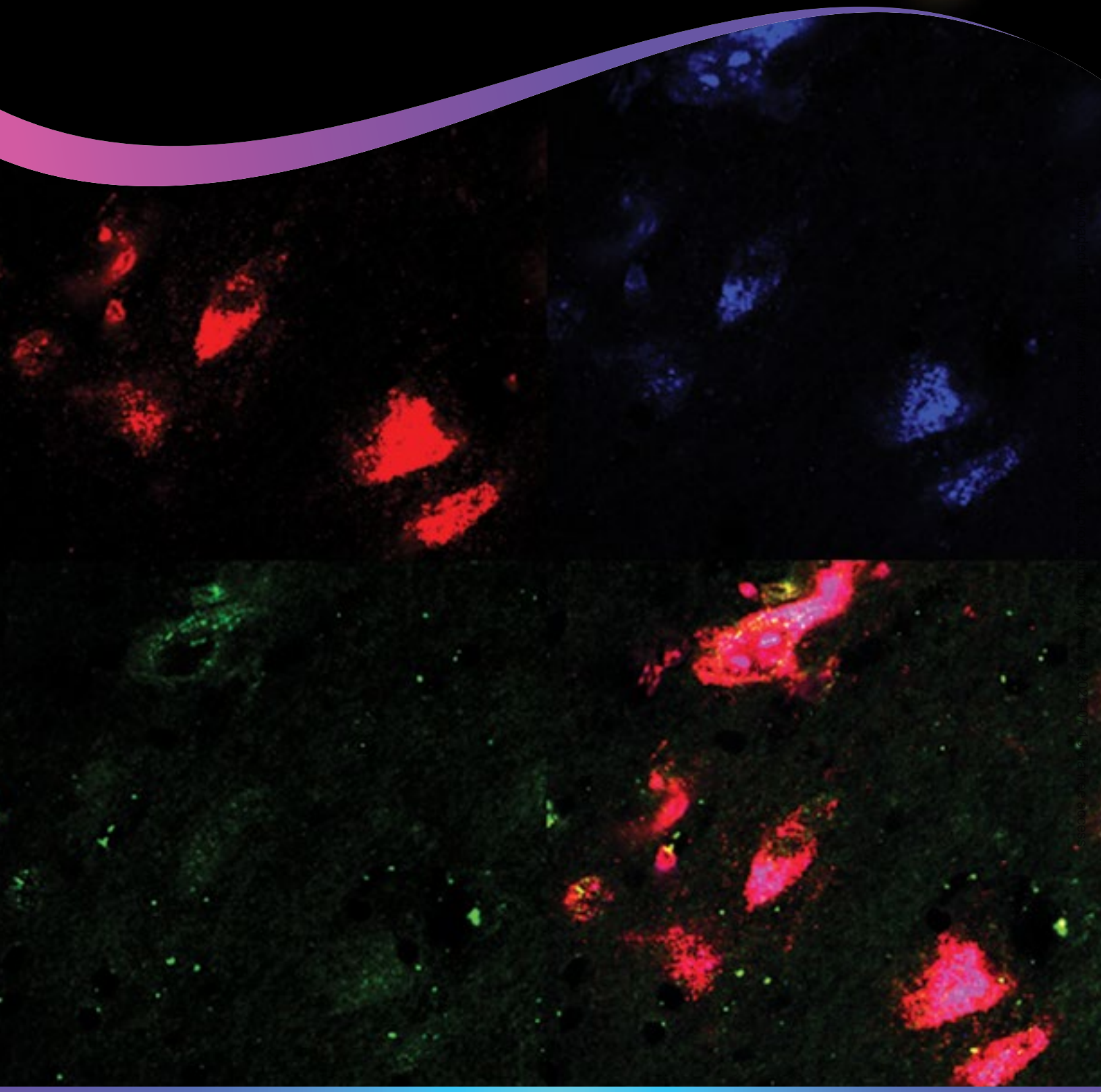

Volume 1 | Number 3 | May 2025

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Genomic Psychiatry

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Cover Art

Insets with TH mRNA+ neurons in red, either single labeled, or co-labeled with VGluT2 (blue) and/or GAD1 (green) mRNA. Simple arrow = single labeled TH+ neurons, arrowhead = TH/VGluT2+ neuron, double arrowhead = triple labeled TH/VGluT2/GAD1 neurons (not all cells are labeled for simplicity). This cover image corresponds to Figure 6C in the paper "Translating stress systems: corticotropin releasing factor (CRF), its receptors, and the dopamine system in nonhuman primate models" by Julie L. Fudge et al. on pages 28–43 in this issue.

Image credit: Julie L. Fudge.

This issue is now available at https://url.genomicpress.com/2p8njsrw.

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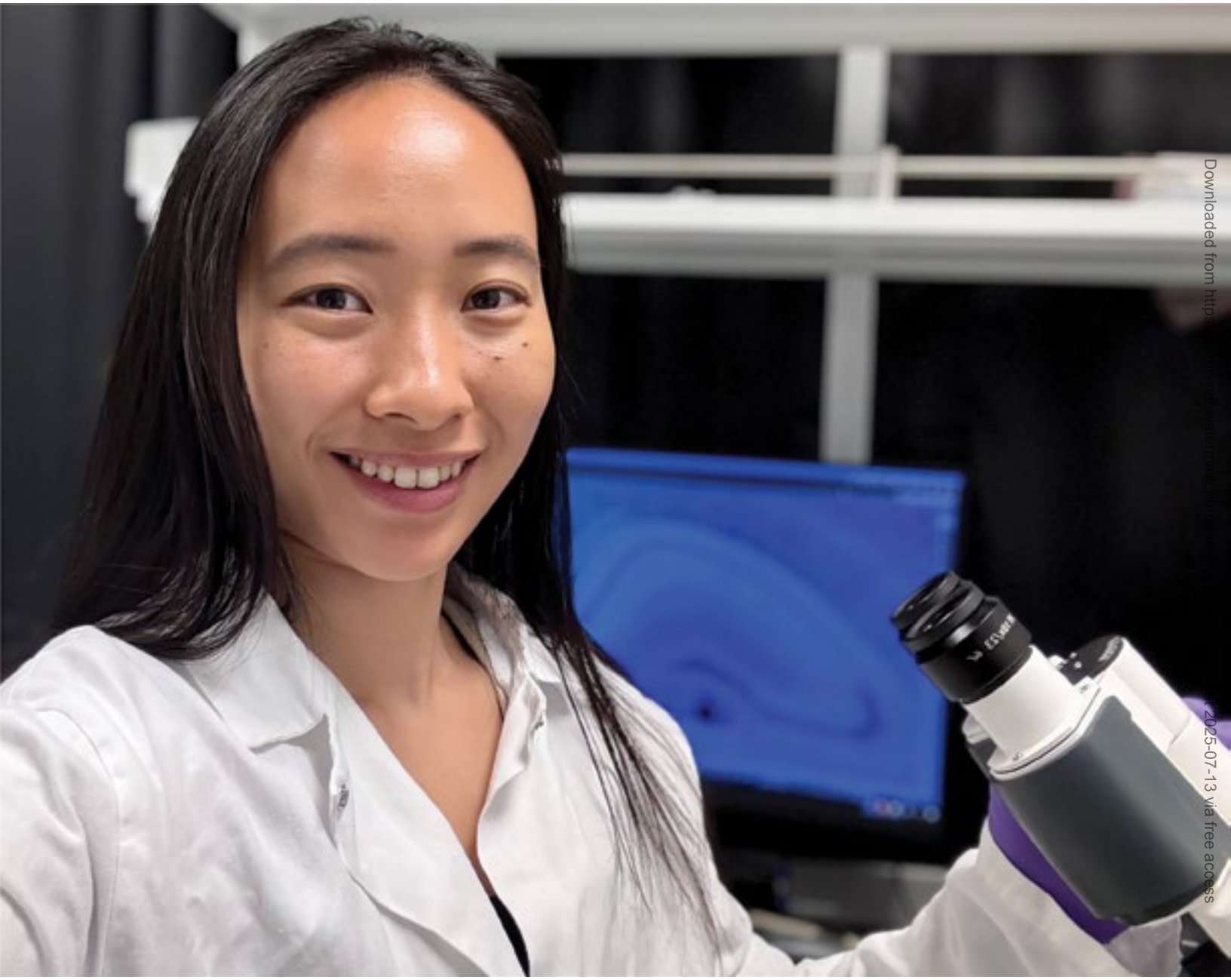

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Featured Rising Star Sophia Shi: https://www.doi.org/10.61373/bm025k.0074

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EDITORIAL

The forgotten clockwork of the brain: Untangling accelerated aging in substance use disorders

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When the scaffolding of biology is hurried by pathology, we are forced to confront time, not as chronology but as degeneration. The elegant study by Kluwe-Schiavon et al. plunges into precisely this conceptual breach: where substance use disorders (SUDs) hijack the natural rhythm of aging, pushing the clock forward with biochemical violence and neuroepigenetic insistence (1). The new article buils on a body of work in this area (2–4). This is not just a question of whether drugs kill. We already know they do. The deeper question, provocative and new, thanks to this anatomically grounded work, is whether drugs age the brain (5). And if so, how (see Fig. 1). There is also a quieter dimension here: one that lives outside the elegant research presented in this article. In this same issue of *Genomic Psychiatry*, a personal interview with Dr. Consuelo Walss-Bass sheds light on the emotional and intellectual backdrop to this research (6).

With rigor and restraint, the authors dissect the transcriptomic and epigenetic landscapes of the dorsolateral prefrontal cortex (DLPFC)—a brain region central to decision-making and executive control, but also particularly vulnerable to the long shadows cast by addiction. Using post-mortem brain tissue from individuals with alcohol, opioid, and stimulant use disorders, the authors deploy not one but three specialized epigenetic clocks calibrated for cortical tissues. These include DNAmClock-Cortical, CerebralCortexClockcommon, and PCBrainAge; each of them represents a fine-grained chronometer that ticks not with seconds, but with methylation.

The central insight of the study is unsettling in its clarity: individuals with SUDs exhibit signs of accelerated biological aging, and this aging is neither cosmetic nor metaphorical. It is cellular. It is molecular. And it is coded into the methylated terrain of the genome (7, 8). That these effects were observed specifically in the brain—rather than peripheral tissues—deepens their clinical gravity. We are not speaking here of graying hair or stiffening joints, but of the cognitive architectures that underlie judgment, memory, and behavioral restraint.

Figure 1. The biological clock of addiction. This conceptual image illustrates the central theme of accelerated biological aging in substance use disorders. A human brain model positioned alongside an analog clock and substance residue (cocaine) visually represents how substance use disorders can accelerate the biological aging process of neural tissue, highlighting the "ticking clock" metaphor discussed throughout the editorial. Image generated by Grok (xAI, 2025) with active author input.

What the Data Whispered

The authors' analytical choreography is both sophisticated and honest. Samples were stratified into those with and without accelerated aging (AA), allowing for within-cohort comparisons that illuminate rather than blur. The transcriptomic profiles revealed overlapping and unique gene expression changes across SUD subtypes. These alterations were not vague or diffuse. They were concentrated in specific biological pathways: mitochondrial function, cellular metabolism, immune modulation, and neuroinflammation (9).

Of particular interest is the mitochondrial signature that emerges across all SUDs, suggesting a shared mechanism of neuroenergetic decay (10). If mitochondria are indeed the powerhouses of the cell, then substance use seems to be the arsonist. The implication is grim: that addiction robs the brain of its metabolic youth.

Equally fascinating is the differential enrichment across substance types. For instance, alcohol and stimulants shared vascular and oxygen transport system disruptions, while opioids and stimulants converged on inflammatory pathways. Alcohol and opioids, in contrast, intersected within cellular signaling and neurodevelopmental tracks. These divergences underscore a point that psychiatry often ignores in its pharmacological zeal: that not all addictions are created equal at the molecular level. There is no "one SUD to rule them all"—only overlapping morbidities traversing unique biological corridors.

Bravery in Limitation

The authors, commendably, resist the temptation of over-interpretation. They acknowledge the limitations inherent to cross-sectional post-mortem studies. They admit the absence of causality, the specter of confounding, the constraints of nominal significance thresholds. Most notably, they point out that no differentially expressed genes (DEGs) survived false discovery rate (FDR) correction, a humbling reminder of the statistical rigor demanded by genomic inquiry.

Yet, science often advances not through definitive answers, but through the elegance of an intelligent question. And this study asks many—quietly but insistently. Why do some brains crumble faster than others under the same pharmacological siege? Could there be predisposing genomic signatures: either genetic susceptibilities or epigenetic scars left by early-life adversity, that make some individuals biologically fragile to the insult of drugs? What role might immune priming,

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neurovascular shifts, or hormonal derangements play in this neurobiological acceleration?

The Policy Reverberations

It would be a mistake to leave this study in the quietude of the laboratory. Its implications are vast, reaching into public health, addiction medicine, criminal justice, and even education policy. If substance use induces premature biological aging, then we must treat it not merely as a moral lapse or behavioral choice, but as an accelerant of neurodegeneration. What we call relapse may sometimes be the cognitive exhaustion of a prematurely aged cortex. What we term non-adherence might instead be mitochondrial collapse.

In an era that fetishizes longevity and "healthspan," it is almost tragicomic that we ignore entire populations whose biological age far outpaces their years. Youth, in the statistical sense, is no shield when the brain is decades older than the body it inhabits.

A Call Forward

This study opens the door to a field that remains embryonic but urgent: the psychiatry of aging in young people. It calls for longitudinal investigations that follow individuals through abstinence, relapse, remission, and decay. It demands integrative biomarker panels that combine methylation, gene expression, and neuroimaging. It proposes, albeit implicitly, a new taxonomy for SUD, not just based on behavior or drug class, but on biological decay signatures.

If one is to be optimistic, and one must be, even in the face of molecular entropy, then perhaps these findings mark the beginning of a therapeutic redirection. Anti-aging interventions, long the obsession of cosmetic medicine and Silicon Valley biohackers, might soon find their most ethically urgent application in addiction psychiatry.

Julio Licinio[1]

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References

1. Kluwe-Schiavon B, Stertz L, Barichello T, Meyer TD, Fries GR, Walss-Bass C. Deciphering the molecular basis of accelerated biological aging in substance use disorder: integrative transcriptomic analysis. Genomic Psychiatry. 2025. DOI: 10.61373/gp025a.0029.
2. Zillich L, Cetin M, Hummel EM, Poisel E, Fries GR, Frank J, et al. Biological aging markers in blood and brain tissue indicate age acceleration in alcohol use disorder. Alcohol Clin Exp Res (Hoboken). 2024;48(2):250–9. DOI: 10.1111/acer.15241. PMID: 38276909; PMCID: PMC10922212
3. Cabrera-Mendoza B, Stertz L, Najera K, Selvaraj S, Teixeira AL, Meyer TD, et al. Within subject cross-tissue analyzes of epigenetic clocks in substance use disorder postmortem brain and blood. Am J Med Genet B Neuropsychiatr Genet. 2023;192(1-2):13–27. DOI: 10.1002/ajmg.b.32920. PMID: 36056652; PMCID: PMC9742183
4. Bachi K, Sierra S, Volkow ND, Goldstein RZ, Alia-Klein N. Is biological aging accelerated in drug addiction? Curr Opin Behav Sci. 2017;13:34–9. DOI: 10.1016/j.cobeha.2016.09.007. PMID: 27774503; PMCID: PMC5068223
5. Ersche KD, Jones PS, Williams GB, Robbins TW, Bullmore ET. Cocaine dependence: a fast-track for brain ageing? Mol Psychiatry. 2013;18(2):134–5. DOI: 10.1038/mp.2012.31. PMID: 22525488; PMCID: PMC3664785
6. Walss-Bass C. Consuelo Walss-Bass: why does my sister have schizophrenia and I do not? Understanding how a person's unique genetic makeup interacts with their environment to shape behavior is one of the final frontiers in medicine. Genomic Psychiatry. 2025:1–4. DOI: 10.61373/gp025k.0014
7. Clark SL, McGinnis EW, Zhao M, Xie L, Marks GT, Aberg KA, et al. The impact of childhood mental health and substance use on methylation aging into adulthood. J Am Acad Child Adolesc Psychiatry. 2024;63(8):825–34. DOI: 10.1016/j.jaac.2023.10.014. PMID: 38157979; PMCID: PMC11745081
8. Onisiforou A, Zanos P, Georgiou P. Molecular signatures of premature aging in Major depression and Substance Use disorders. Sci Data. 2024;11(1):698. DOI: 10.1038/s41597-024-03538-z. PMID: 38926475; PMCID: PMC11208564
9. Li X, Ramos-Rolón AP, Kass G, Pereira-Rufino LS, Shifman N, Shi Z, et al. Imaging neuroinflammation in individuals with substance use disorders. J Clin Invest. 2024;134(11). DOI: 10.1172/JCI172884. PMID: 38828729; PMCID: PMC11142750
10. Reece AS. Evidence of accelerated ageing in clinical drug addiction from immune, hepatic and metabolic biomarkers. Immun Ageing. 2007;4:6. DOI: 10.1186/1742-4933-4-6. PMID: 17892544; PMCID: PMC2041948

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INNOVATORS & IDEAS: RISING STAR

Jeremie Poschmann: Data-driven discovery in human diseases through multi-omics profiling of the circulating immune system

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Keywords: Multi-omics, circulating immune system, data-driven discovery, disease heterogeneity, immune profiling, patient cohorts

Dr. Jeremie Poschmann leads a research group at INSERM and Université de Nantes, where he investigates the human immune system with a focus on the circulating immune compartment. His work combines multi-omics and data-driven approaches to uncover immune mechanisms that influence disease susceptibility and patient outcomes, particularly in infectious and psychiatric conditions. Trained originally as a nurse, Dr. Poschmann entered science driven by a deep curiosity for the unresolved complexities of human biology. His career has taken him through Germany, Belgium, Canada, Singapore, the UK, and France, shaping his collaborative and cross-disciplinary mindset. A self-taught bioinformatician, he values independence in research and actively fosters a diverse and inclusive team. In this Genomic Press Interview, he reflects on pivotal moments in his journey including an early fascination with genome-wide discovery and shares how pre-existing immune states may help explain why individuals respond differently to disease exposure. Outside the lab, he finds balance through surfing and chess, which keeps his thinking sharp. Committed to translating research into real-world impact, Dr. Poschmann is equally passionate about mentoring emerging scientists and building a culture that supports innovation and integrity.

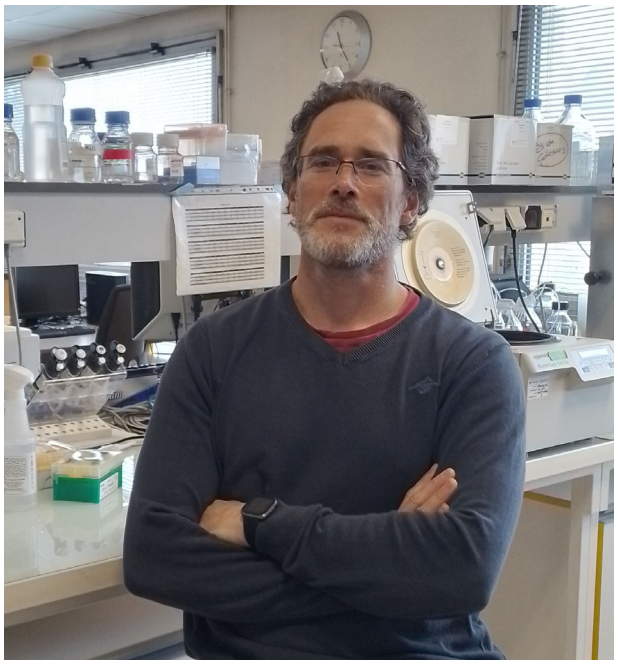

Figure 1. Jeremie Poschmann, PhD, Université de Nantes, France.

Part 1: Jeremie Poschmann – Life and Career

Could you give us a glimpse into your personal history, emphasizing the pivotal moments that first kindled your passion for science?

I was not especially interested in science in school. To be honest, I was a bit lazy and not particularly curious at the time. But I always liked mathematics. I was drawn to its clarity and how you could arrive at a solution that stood on its own, regardless of who you were or what you believed.

Things changed when I entered nursing school. I realized that the more I asked, the more there was to uncover and that, for all we know, so much remains unexplained. That moment of realization sparked something in me. Curiosity took over, and I found myself wanting to dig deeper. That was the beginning of my path to research.

We would like to know more about your career trajectory leading up to your current role. What defining moments channeled you toward this opportunity?

One of the earliest pivotal moments in my career was realizing the power of discovery research through "omics" profiling. I was working on ChIP-on-chip experiments studying RNA polymerase II in yeast, before NGS was available. I was struck that a single experiment could give you a genome-wide view of what was happening in the cells. From that data alone, I could start forming hypotheses. That ability to follow the data rather than impose a question on it was deeply compelling.

Another shift for me was when NGS became available. Suddenly, we could do genome-wide ChIP in humans in a single experiment. That blew my mind. I remember thinking, "*This is it—this is what I want to do.*" It was clear to me that the future of biology was here, and I was determined to be at the forefront of it.

The final turning point came during my postdoc. Each experiment generated massive amounts of data, and I constantly waited for bioinformaticians to analyze it. The delays were frustrating, and I realized they were not necessarily as driven as I was to analyze my data. So, I decided to take matters into my own hands. I began learning coding and data analysis independently, teaching myself the computational side of the work. I was fortunate to be surrounded by a strong bioinformatics environment supporting this transition. Looking back, that decision was transformative. It allowed me to run my projects independently, from experimental design to analysis and interpretation. This laid the foundation for starting my own lab with full independence.

Please share with us what initially piqued your interest in your favorite research or professional focus area.

What hooked me early on is the idea that data itself can lead the way. After seeing how much could be uncovered in an unbiased, genome-wide approach, I became fascinated by the potential to let patterns in the data guide the next question rather than relying on predefined hypotheses. It's

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the interplay between exploration and insight where the unexpected becomes visible. There is something uniquely exciting about being able to ask, *"What is the system telling us?"*

What impact do you hope to achieve in your field by focusing on specific research topics?
My journey started in nursing, moved through developing genomic approaches in yeast, and has since progressed to doing omics profiling in patient cohorts. What drives me now is the hope of making it full circle. My dream is that one day, the discoveries I help make will be directly relevant to real patients. Whether identifying molecular signatures, predicting treatment response, or uncovering new mechanisms, I want the science I do to inform care, not just ultimately understanding. That is the dream.

Please tell us more about your current scholarly focal points within your chosen field of science?
My research spans various diseases, from host-pathogen interactions to psychiatric disorders. However, the common thread is the immune system, focusing on the circulating immune compartment. I work with blood because it is accessible and offers a dynamic window into systemic processes. I aim to identify molecular signatures and mechanisms linked to different disease states through a discovery-driven approach using various omics technologies.

A central hypothesis in my work is that pre-existing immune states significantly influence disease susceptibility and outcomes. I am particularly interested in how immune memory-like mechanisms contribute to heterogeneity. For example, why do some individuals exposed to the same pathogen, such as SARS-CoV-2, develop severe illness or die, while others remain asymptomatic or recover easily? These differences reflect a form of environmental imprint, an individual's personal immune history, that we can now begin to explore through functional genomics.

What habits and values did you develop during your academic studies or subsequent postdoctoral experiences that you uphold within your research environment?
Research has taken me across continents, from becoming a Nurse in Germany and studying in Belgium to doing my PhD in Canada, a postdoc in Singapore, and working in the UK before settling in France. Each place has taught me something different, not just scientifically but also in terms of how people think, collaborate, and approach challenges. I have learned to truly value those cultural differences and the distinct ways of doing things that come with them.

In my group, I foster that same openness. Diversity makes science stronger. I do not necessarily choose team members based on grades, I look for what makes someone unique, their experiences, mindset, and how they think differently. Those qualities greatly contribute to a more dynamic and original research environment.

At Genomic Press, we prioritize fostering research endeavors based solely on their inherent merit, uninfluenced by geography or the researchers' personal or demographic traits. Are there particular cultural facets within the scientific community that warrant transformative scrutiny, or is there a cause within science that deeply stirs your passions?
One profoundly troubling issue is the growing divide between permanent and non-permanent scientific positions in France and elsewhere. Increasingly, only principal investigators hold permanent roles, while postdocs, engineers, and technicians are hired on short-term contracts, often with very little security or long-term prospects. As a PI, this makes it incredibly difficult to retain talented people, not because of work or science, but because of structural instability and chronic underfunding.

This goes against the very nature of research. It is a team effort. Progress depends on continuity, shared expertise, and trust built over time. We should invest in our teams, not cycle through them. I believe strongly that if we want sustainable, high-quality science, we need to offer stability and recognition to *everyone* contributing to the work, not just those at the top.

What do you most enjoy in your capacity as an academic or research rising star?
Mentoring has become one of the most rewarding aspects of academic life. Watching students grow from their master's projects to their Ph. D.s and beyond is incredibly fulfilling. It's not just about guiding them scientifically but about seeing their confidence, independence, and curiosity evolve over time.

Outside professional confines, how do you prefer to allocate your leisure moments, or conversely, in what manner would you envision spending these moments given a choice?
Outside of the lab and family life, surfing is where I find the most joy and balance. It is physically demanding and mentally absorbing. Surfing requires grit, patience, endurance, and strength. You paddle hard, sometimes for nothing, but then a perfect set rolls in, and you must be ready to catch it. That mix of challenge and anticipation keeps me sharp and grounded. Also, being surrounded by nature out in the water, away from everything, offers a perspective I don't find anywhere else. It clears my mind, resets my energy, and reminds me to stay connected to the moment.

When I am not in the water, I also enjoy playing chess. It is a different kind of focus, but I can get fully immersed in it. Chess helps me slow down and shift into a reflective mindset. Each position requires me to reevaluate my prior decisions. I like the balance it demands between intuition and calculation.

Part 2: Jeremie Poschmann – Selected questions from the Proust Questionnaire[1]

What is your idea of perfect happiness?
For me, it's something as simple as having a picnic with my family at the beach when we're traveling together, or being alone in the ocean, sitting on my surfboard, watching the sunset.

What is your greatest fear?
Right now, my greatest fear is the global rise of fascism and autocracy, while environmental issues continue to be neglected.

Which living person do you most admire?
Volodymyr Zelenskyy, for the way he transformed from a comedian into a steadfast defender of his people. I deeply admire his courage, adaptability, innovation, and strong sense of responsibility in the face of relentless pressure.

What is your greatest extravagance?
Taking a weekend off to go surfing on my own.

What are you most proud of?
That I have passed on my passion for travel and foreign cultures to my kids.

[1] In the late nineteenth century, various questionnaires were a popular diversion designed to discover new things about old friends. What is now known as the 35-question Proust Questionnaire became famous after Marcel Proust's answers to these questions were found and published posthumously. Proust answered the questions twice, at ages 14 and 20. In 2003 Proust's handwritten answers were auctioned off for $130,000. Multiple other historical and contemporary figures have answered the Proust Questionnaire, including among others Karl Marx, Oscar Wilde, Arthur Conan Doyle, Fernando Pessoa, Stéphane Mallarmé, Paul Cézanne, Vladimir Nabokov, Kazuo Ishiguro, Catherine Deneuve, Sophia Loren, Gina Lollobrigida, Gloria Steinem, Pelé, Valentino, Yoko Ono, Elton John, Martin Scorsese, Pedro Almodóvar, Richard Branson, Jimmy Carter, David Chang, Spike Lee, Hugh Jackman, and Zendaya. The Proust Questionnaire is often used to interview celebrities: the idea is that by answering these questions, an individual will reveal his or her true nature. We have condensed the Proust Questionnaire by reducing the number of questions and slightly rewording some. These curated questions provide insights into the individual's inner world, ranging from notions of happiness and fear to aspirations and inspirations.

Figure 2. Jeremie Poschmann after a surfing session on France's Atlantic coast, where he finds balance away from the laboratory. This passion reflects his philosophy of combining scientific rigor with personal renewal, as he describes surfing as requiring "grit, patience, endurance, and strength" – qualities that mirror his approach to scientific discovery.

What is your greatest regret?
I wasted a few years as a teenager and young adult, not knowing what I wanted and drifting without direction. Looking back, I wish I had used that time more intentionally.

What is the quality you most admire in people?
Unprovoked compassion.

What is the trait you dislike most in people?
Self-centered, me-first mindset.

What do you consider the most overrated virtue?
Intelligence

What is your favorite occupation (or activity)?
Surfing and playing chess.

Where would you most like to live?
Somewhere I can surf and do science in the same day.

What is your most treasured possession?
I don't know: I am not very attached to material things.

When and where were you happiest? And why were so happy then?
When I am in the zone, writing, thinking, or focusing deeply.

What is your current state of mind?
Focus on what I can change and leave the rest aside.

What is your most marked characteristic?
I can hold strong opinions but also change them quickly if the arguments change.

Among your talents, which one(s) give(s) you a competitive edge?
I am good at playing chess, and logical deduction and calculating options give me an edge in scientific reasoning and interpreting results.

What do you consider your greatest achievement?
Getting myself up from a lazy teenager to becoming a scientist.

If you could change one thing about yourself, what would it be?
To find the correct answer immediately, not at three in the morning.

What do you most value in your friends?
Friendly competition.

Who are your favorite writers?
Terry Pratchett.

Who are your heroes of fiction?
Sam Vimes, a Terry Pratchett character. He is a cynical but deeply moral policeman who always does the right thing, even when it is hard or thankless. He stands for justice in a corrupt world.

Who are your heroes in real life?
Ludovico Einaudi, for creating music that is both beautiful and deeply moving.

Jonathan Mill, for combining strong scientific work with real kindness and respect for his team members.

Jay Shendure, for his brilliant ideas and major contributions to molecular biology and genomics.

What aphorism or motto best encapsulates your life philosophy?
Daily action makes progress.

Better done than perfect.

Nantes, France
7 April 2025

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INNOVATORS & IDEAS: RESEARCH LEADER

Melissa Perreault: Thinking big towards a "complexity science" approach in neuroscience – systems, environment, and whole organism research

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Keywords: Neuropsychiatric disorders, neuroethics, systems neurobiology, drug discovery, complexity science, Indigenous

Dr. Melissa Perreault, Professor in the Department of Biomedical Sciences at the University of Guelph and member of the College of New Scholars, Artists, and Scientists in the Royal Society of Canada, is participating in the Genomic Press Interview, sharing her unique insights and experiences. As a neuroscientist and citizen of the Métis Nation of Ontario, Dr. Perreault's work bridges Indigenous perspectives with Western neuroscience, focusing on elucidating sex-specific neurobiological mechanisms underlying neuropsychiatric and neurodevelopmental disorders. Her research aims to identify novel biomarkers and therapeutic targets. At the same time, her advocacy extends to promoting Indigenous representation in STEM fields through initiatives like the Indigenous STEM Mentorship Program at the University of Guelph. Dr. Perreault's efforts also encompass promoting ethical engagement with Indigenous communities in neuroscience research globally, championing the integration of Indigenous knowledge into brain science through international collaborations. Her multifaceted approach to neuroscience, combining rigorous scientific inquiry with cultural sensitivity and inclusivity, positions her at the forefront of a new era in brain research that embraces diverse perspectives and holistic understanding.

Part 1: Melissa Perreault – Life and Career

Could you give us a glimpse into your personal history, emphasizing the pivotal moments that first kindled your passion for science?

Neither of my parents finished secondary school. I was raised in a one-parent home, and my mother worked odd jobs to supplement being on social assistance. I had a good childhood, though. We rented a small home near a forest, and I spent a lot of time there. I remember wanting to be a medical doctor from a very young age, though I cannot say whether a specific moment or event triggered my passion for the field. But I do remember thinking that as I got older and understood the family situation better, my life would be different from that of my mother's, and I would grow up to be a financially independent woman. I never expected that trying to achieve that goal would be challenging.

I went into the honors Biology program at McMaster University in Ontario. I loved biology; it was a good program that would get me closer to becoming a medical doctor. It was not easy being a first-generation university student. Still, it was not until I heard the stories and experiences of my classmates that I realized how much I was at a disadvantage. I was unaware of awards or scholarships to apply for, and I did not know whether any academic support was available or how to access them. But I persevered, and I did well, all things considered. In my last year of university, I was first introduced to research in my thesis project. I worked in an evolutionary biology lab focusing on growth and reproductive tradeoffs with Dr. C. David Rollo. It was here that I found my passion for research. I realized then that although I had thought I wanted to be a medical doctor, I was happiest when I had the opportunity to ask innovative scientific questions and be able to take steps toward finding the answer.

Figure 1. Melissa Perreault, Ph.D., University of Guelph, Canada.

I stayed for an MSc in the same lab but did not receive a stipend, only a teaching assistantship that paid for my tuition, so I supplemented my income by working the night shift on weekends at a donut shop. I loved my lab and project, but when I finished with my degree, I was not sure what to do. I worked for a wildlife removal company for a time, got married, and had my first child. I eventually went into radiation therapy school to treat cancer patients but did not feel I was stimulated enough in such a role. It was excellent timing that my past MSc supervisor reached out to try and coerce me back to graduate school for a PhD as he felt I had a strong aptitude for research. He had a colleague who worked in the Psychiatry and

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Behavioural Neurosciences department, Dr. Henry Szechtman, who happened to be looking for a student. It was my first exposure to translational behavioral neuroscience research. I still do translational neuroscience research to this day.

My PhD project used a rat model to study obsessive-compulsive disorder. I had the opportunity to collaborate with excellent scientists including Dr. Philip Seeman and Dr. Jane Foster. Working in Jane's lab introduced me to molecular and cellular neurobiology, and I really enjoyed this mechanistic research. I decided that when I completed my PhD, I wanted to continue to build on these skills.

In my last year of my PhD training, I had my second child and then joined Dr. Susan George's laboratory at the University of Toronto shortly after graduating. Her expertise in neuropharmacology and her focus on dopamine signaling was an excellent fit for me. It was also during my PhD training that my father told me I was Indigenous. I sat with that information for a long time as I was not sure what to do with it.

We would like to know more about your career trajectory leading up to your most relevant leadership role. What defining moments channeled you toward that leadership responsibility?

My postdoctoral training with Dr. George was highly beneficial to my career. I not only had the opportunity to learn about neuropharmacology, I learned what it was like to be part of a team. I had independence and leadership opportunities. She played an important role in the development of the person I am today, and I am grateful for all of the opportunities that she provided. Unfortunately, despite having several high-impact publications and what I believed to be a strong skill set and good ideas, I could not find employment as an independent investigator. It took over eight years.

There were two defining moments in my training, one that gave me an edge toward scientific independence and one that hindered my progress. Through a scholarship from the Canadian Institutes of Health Research, I had the opportunity to spend three weeks in Italy attending the Neuroscience School of Advanced Studies. There, I met Dr. Anthony Grace, an instructor for the course. I subsequently spent some time in Tony's lab learning systems electrophysiology, which provided an additional approach I could use in my program. Tony was also a research mentor for my application for a NARSAD Young Investigator Award, which I was successful in attaining. He had a significant impact on my career progression. He was not only a mentor, but he became a dear friend.

Outside of my research, I began looking into my Indigenous lineage. I spent time at Indigenous events and connected with my Métis culture. I kept this side of myself apart from my work. It was a different time, and being Indigenous was not something you announced. To my knowledge, there was no one like me, which is not surprising given the extremely low representation of Indigenous peoples in neuroscience. As mentioned, I had been applying for faculty positions for years and had yet to get a single interview. I was discussing my frustration with a senior faculty member one day when she asked me if I had my Indigeneity on my applications. I told her I did. She responded that I should remove it. I cannot say conclusively whether removing my Indigeneity from my applications was directly responsible for the interviews I was suddenly able to get. I have thought a lot about it over the years.

I began at the University of Guelph in 2017. I had lost so much time that I had catching up to do. Combining all my technical skills, I was able to develop a successful research program. I excelled at my job and was promoted to Associate Professor with tenure in 2021. However, research was not all I was doing. I developed the Indigenous STEM Mentorship Program and received funding to develop other supports for Indigenous students and to Indigenize the science building through art and renaming. I found a strong ally in Dr. T. Ryan Gregory, who supported me and my initiatives. My efforts in this domain began to be noticed by people who wanted to learn. I was approached by hospitals, universities, and neuroscience organizations to educate on decolonization, Indigenization, and Indigenous capacity building. I was also recruited by the Canadian Brain Research Strategy to be on the Indigenous Knowledge Holder's Advisory Group. In this role, I had (and still do!) the privilege of working with Dr. Judy Illes from the University of British Columbia, who was also Director of Neuroethics Canada and Chair of the International Brain Initiative. Judy allowed me to expand on my initiatives, build collaborations, and start working towards integrating Indigenous knowledge into neuroscience on a global scale.

Today, as a University of Guelph Research Leadership Chair, I balance a full multidisciplinary research program in translational neuroscience with my work in neuroethics and my Indigenous initiatives. It is difficult, but after many years, I believe that I have finally managed to successfully combine the neuroscientist with the Indigenous woman.

Please share with us what initially piqued your interest in your favorite research or professional focus area.

Over the course of my career, I identified important knowledge gaps not only in the neuroscience research we do but also in how we do it. I also experienced a general lack of cultural humility that was particularly pronounced in neuroscience regarding valuing other knowledge and world views. These realizations slowly led to the development of three general research areas that I work on today. The first is the identification of sex-specific biomarkers and therapeutic targets in neuropsychiatric and neurodevelopmental disorders with the goal of moving the field toward more personalized medicine. My second focus is on "complexity science," or holistic research. Though there have been substantial benefits to using reductionistic approaches in neuroscience, there needs to be a better understanding of how neuroscience fits into the broader picture. My final focus is neuroethics, where I educate on ethical Indigenous community research approaches and advocate for integrating Indigenous ways of knowing and doing into neuroscience.

What impact do you hope to achieve in your field by focusing on specific research topics?

I hope scientists will begin to take more real-world approaches to solve real-world problems. This can be achieved through inclusivity in what we study and how we study it. For example, there has been significant growth in whole plant and mushroom use by the public, and this use will only continue to grow in an era of climate change, pandemics, and economic and political uncertainties. These organisms contain hundreds of molecules, many of which are bioactive. As researchers, however, we rarely study the whole organism, with a predominant focus on the major chemical constituents. Entourage effects are overlooked, contraindications with prescription medications are not examined, and the therapeutic value of low-abundance molecules is, for the most part, ignored. Traditional uses for these plants, knowledge acquired over millennia and validated through experience, are also rarely acknowledged. Other examples include the recent widespread acceptance of the need to include sex and gender in neuroscience research or the acknowledgment of the importance of other organ systems on brain health.

From an Indigenous community research perspective, strength-based approaches that are inclusive of communities as research partners instead of research subjects are only now being embraced following a long history of exploitation and stereotyping, and this is by no means restricted to Indigenous communities. We have a long way to go, but perceptions are changing, and alternative views and approaches to our science and how we do it are becoming more accepted.

Please tell us more about your current scholarly focal points within your chosen field of science.

My translational research currently centers around sex-specific biomarkers and therapeutic target identification in depression and autism spectrum disorders, the role of infection in maternal immune activation and its impacts on neurodevelopment, and the neurobiological effects of whole psilocybin mushrooms and other traditional medicines. I also work with Indigenous and non-Indigenous researchers from across the globe on ethical engagement with Indigenous communities in neuroscience research.

Figure 2. Dr. Perreault loves scuba diving in her spare time. Although there are many things to see under the ocean, sharks are among the most exciting creatures. Here, she is diving in the Bahamas with black-tip reef sharks.

What habits and values did you develop during your academic studies or subsequent postdoctoral experiences that you uphold within your research environment?
In academia, rejection is common at all training and career stages. I talk about my rejections with my trainees and how I do not let them affect me. Until we reach such a stage where there are more scientific resources, it is my responsibility to help my trainees develop resilience to rejection and to help them understand that rejection does not equal failure. I have also always been averse to the idea of "imposter syndrome". Coming from a smaller institution, I always talk to my trainees about academic privilege and how not to compare themselves to those with more resources and support systems.

At Genomic Press, we prioritize fostering research endeavors based solely on their inherent merit, uninfluenced by geography or the researchers' personal or demographic traits. Are there particular cultural facets within the scientific community that warrant transformative scrutiny, or is there a cause within science that deeply stirs your passions?
Three things require more transformative scrutiny. First, we need to take more time to evaluate impactful science for scholarships, awards, grants, promotions, etc. Only by taking this time can we promote scientific quality over quantity. Second, we need to start expressing cultural humility, recognizing the value of traditional knowledge and world views, and appreciating that embracing multiple perspectives puts us in a better position to develop new connections and ask more innovative scientific questions. Lastly, we need to start studying plants and mushrooms in the way that they are being used in a population. We need to invest in infrastructure to ensure that the relative abundance of the chemical constituents in these organisms is maintained so studies can be replicated and entourage effects can be considered.

What do you most enjoy in your capacity as an academic or research leader?
For me, it is all about building connections with my trainees, colleagues, and those from the community that I have the privilege of meeting. I also enjoy knowing that my work will make a difference to those who come after me.

Outside professional confines, how do you prefer to allocate your leisure moments, or conversely, in what manner would you envision spending these moments given a choice?
If it were possible, I would spend every free moment on a beach by the ocean in a tropical location. I scuba dive when I am able, and I am somewhat of a shark chaser. I have a motorcycle that I ride to help me unwind in the summer, but I also spend time hiking or at the gym for exercise. I am a fan of science fiction and fantasy books and settle in to read when I have the opportunity.

Part 2: Melissa Perreault – Selected questions from the Proust Questionnaire[1]

What is your idea of perfect happiness?
I am still trying to figure that out.

[1]In the late nineteenth century, various questionnaires were a popular diversion designed to discover new things about old friends. What is now known as the 35-question Proust Questionnaire became famous after Marcel Proust's answers to these questions were found and published posthumously. Proust answered the questions twice, at ages 14 and 20. In 2003 Proust's handwritten answers were auctioned off for $130,000. Multiple other historical and contemporary figures have answered the Proust Questionnaire, including among others Karl Marx, Oscar Wilde, Arthur Conan Doyle, Fernando Pessoa, Stéphane Mallarmé, Paul Cézanne, Vladimir Nabokov, Kazuo Ishiguro, Catherine Deneuve, Sophia Loren, Gina Lollobrigida, Gloria Steinem, Pelé, Valentino, Yoko Ono, Elton John, Martin Scorsese, Pedro Almodóvar, Richard Branson, Jimmy Carter, David Chang, Spike Lee, Hugh Jackman, and Zendaya. The Proust Questionnaire is often used to interview celebrities: the idea is that by answering these questions, an individual will reveal his or her true nature. We have condensed the Proust Questionnaire by reducing the number of questions and slightly rewording some. These curated questions provide insights into the individual's inner world, ranging from notions of happiness and fear to aspirations and inspirations.

What is your greatest fear?
Starting so late as an independent investigator, I worry I will never be able to afford to retire.

What is your greatest extravagance?
My annual vacation with my adult children.

What are you most proud of?
Against all odds, getting my PhD.

What is the quality you most admire in people?
Authenticity.

What is the trait you most dislike in people?
Dishonesty.

What is your favorite occupation (or activity)?
I have the best occupation.

Where would you most like to live?
I dream about owning a home close to the ocean on a tropical island.

What is your most treasured possession?
My memories and joyful experiences.

When and where were you happiest? And why were so happy then?
I was scuba diving off the coast of Barbados and found myself in a school of small squid. I was diving with two others, but they were not nearby as they were doing training, and so I was alone. It was so peaceful and awe-inspiring, a feeling of pure contentment.

What is your most marked characteristic?
Resilience.

Among your talents, which one(s) give(s) you a competitive edge?
Perseverance. I have overcome many barriers to achieve success. I never give up, and I don't sweat the small stuff.

What do you consider your greatest achievement?
My children.

If you could change one thing about yourself, what would it be?
I would be less shy around new people.

What do you most value in your friends?
That they listen, are honest, and are supportive.

Who are your favorite writers?
Steven Erikson and Brandon Sanderson.

Who are your heroes of fiction?
Not a hero but more someone I admired. Avasarala from the tv show The Expanse. That woman embodied power.

Who are your heroes in real life?
I do not have any real-life heroes.

What aphorism or motto best encapsulates your life philosophy?
Never give up.

Guelph, Ontario, Canada
20 February 2025

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INNOVATORS & IDEAS: RESEARCH LEADER

Anthony A. Grace: Elucidating the circuitries that underlie schizophrenia and depression may reveal the impact of stress during development and identify novel treatment targets

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After finishing his postdoc at NYU in the Physiology Department under the direction of Rodolfo Llinas, Dr. Grace started as an Assistant Professor of Psychology and Psychiatry at the University of Pittsburgh in the fall of 1985. He was promoted early in the fall of 1991 to Associate Professor of Behavioral Neuroscience and Psychiatry and to Professor of Neuroscience and Psychiatry in July 2003. In September 2010, he was again promoted to Distinguished Professor of Neuroscience and Professor of Psychiatry and Psychology, his current position. He is the Editor-in-Chief of the *International Journal of Neuropsychopharmacology*, the journal of the Collegium Internationale Neuropsychopharmacologicum (CINP, International College of Neuropsychopharmacology). Dr. Grace has offered insights into his personal and professional journey.

Part 1: Anthony Grace – Life and Career

Could you give us a glimpse into your history, emphasizing the pivotal moments that first kindled your passion for science?

When I started my college degree at Allegheny College, I had planned to be premed; however, this was not my passion, and my grades could have been better. However, in the fall of my Sophomore year, I took the course Physiological Psychology and was hooked! I became the lab assistant for the course, teaching rodent surgery to my peers and performing my own experiments. From then on, I made straight As because I had a future for which I was enthused, mainly when I learned I could do this for a living! I did a project for my senior project on the role of dopamine in locomotion, designing and constructing an injection cannula to infuse a GABA agonist into the substantia nigra and measuring rotation. Even though I scored in the 98th percentile in biology and 93rd in psychology in my GREs, after applying to twelve graduate schools I received ten rejections immediately, probably because no one had heard about Allegheny College (a small school with only 1700 students in total). I was accepted to the psychology program at Duke and was on the waiting list in the Pharmacology Department at Yale. It came down to me being at the top of the waiting list and one person to hear from, but as luck would have it, I was accepted! When I arrived, I wanted to work with the scientist who had written a very influential paper that guided my undergraduate project. This was a paper by Walters, Bunney, Roth, and Aghajanian. Coming from a small undergraduate college, I knew nothing about authorship, but I decided to work with the most important person – Judy Walters, the first author! But, of course, she was a postdoc who had left for an independent position, so I went to the second author, Steve Bunney! It turned out that Steve had just finished his residency with George Aghajanian and had started his own lab, so I was his first graduate student and had extensive access to him during my training.

Figure 1. Anthony A. Grace, University of Pittsburgh, USA.

We would like to know more about your career trajectory leading to your most relevant leadership role. What defining moments channeled you toward that leadership responsibility?

When I began in Steve Bunney's lab, I showed early skills for recording from dopamine neurons. I was progressing brilliantly until, all of a sudden, I could not find a single dopamine neuron in my recordings! Unbeknown to me, the postdoc, Lana Skirboll, was performing studies on the effects of chronic haloperidol on the striatum. It turns out that I was accidentally using her treated rats! When I was taking a graduate course from Dr. Gordon Shepherd, I learned about overexcitation-induced depolarization block. This gave me the idea that the dopamine neurons were in a depolarization block due to overexcitation by haloperidol. I found that giving apomorphine, which usually inhibits the neurons, restored activity in the midbrain. This led to my first publication as a first-year graduate student. I had also developed an interest in physiology and setup and was the first to perform *in vivo* intracellular recordings from identified dopamine neurons. I was having fun in the lab, and not knowing what was typically expected of a graduate student, I kept working and publishing, having published 22 papers as a graduate student. My interest in physiology led me to work as a postdoc for Rodolfo Llinas, who is a very talented physiologist and appreciates the philosophical aspects of how the brain works, which I found fascinating. After a short $2\frac{1}{2}$ year postdoctoral

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experience and with many publications under my belt, I applied for and was selected as an Assistant Professor at the University of Pittsburgh, where I stayed for my entire career.

Please share with us what initially piqued your interest in your favorite research or professional focus area.
Studying the brain fascinated me from my initial days in college. Taking a physiological psychology course and finding out that the brain had "parts" with functions, I was hooked! This was an intersection between biology and philosophy, which I found fascinating. It was highly unusual for a sophomore to get into this course, as it was a small course with limited enrollment that was usually reserved for Senior psychology majors. But as luck would have it, the professor in the course had a mental breakdown over the summer, and they hired a new faculty member, Ken King. Because he was new, all psychology majors dropped the course, and only 4 remained. This means we all got to work on our own stereotaxics to learn surgery, stimulation, and lesion techniques. Because I did so well, Dr. King chose me to lead the lab for 3 years in college. I read voraciously, and it was my readings about dopamine and schizophrenia that turned me on to studying a system that was responsible for recognizing reality and its disruption in schizophrenia and has been the focus of my research ever since.

What kind of impact have you hoped to achieve in your field by focusing on your specific research topics?
I chose to work in the field of neuropsychopharmacology for two reasons. First, it was trying to understand the most complex puzzle in the universe – how the brain can take in information, process it, and, through this, understand advanced thought processes. However, beyond the intellectual interest, the possibility of helping people suffering from these devastating disorders for which there were not highly effective treatments was also a significant draw. Also, having a love for electronics since grade school, performing electrophysiological recordings was a way to implement my technical background.

Please tell us about your current or most important scholarly focal points within your chosen field of science.
My primary focus is on the biological bases of psychiatric disorders, with an emphasis on schizophrenia and depression, although I have published on OCD and anxiety. The focus is on the circuitry that underlies disorders, their etiology in terms of development and impacts of stress during development, novel treatment targets, and the potential of prevention in susceptible individuals.

What habits and values did you develop during your academic studies or subsequent postdoctoral experiences that you upheld within your research environment?
A saying I heard first from Dr. Ralph Adams, a pioneer in voltammetry, is "the rat is always right." This is a good mantra to remember when frustrated with experimental outcomes. Indeed, I always tell my students that the results you get that differ from what you expect are the most interesting because they tell you something new about the system. I also let the people working in my lab know that they should contribute to all discussions about our research, that I am not omniscient, and that everyone has a significant contribution. I also believe strongly in trainees "owning" their projects – I do not put them on a segment of a multi-author paper; I prefer that they take ownership of the whole project. I also allow trainees to choose their projects since they are most passionate and productive when working on something that interests them rather than me.

At Genomic Press, we prioritize fostering research endeavors based solely on their inherent merit, uninfluenced by geography or the researchers' personal or demographic traits. Are there particular cultural facets within the scientific community that warrant transformative scrutiny, or is there a cause within science that deeply stirs your passions?
We need to have better outreach to third-world countries and underrepresented minorities. Everyone has unique backgrounds, experiences, and expertise, and bringing those together into an interactive environment is positive for everyone.

What have you most enjoyed in your capacity as an academic or research leader?
I enjoy interacting with colleagues – some of the best ideas come from conversations over dinner or on the beach rather than at a formal seminar. I find that the best scientists are also the most likable people with whom to interact since they are not always trying to prove they are better. I also very much enjoy what Peter Kalivas has called "Neuroscience Philosophers" – those individuals who think beyond the box and relate their findings to the "big picture" – i.e., what is likely transformative in how we think of the brain.

Outside professional confines, how do you prefer to allocate your leisure moments, or conversely, in what manner would you envision spending these moments given a choice?
There are several things I enjoy. One passion that I developed early on was photography. Indeed, in high school, my two close friends and I worked for a professional photographer and photographed weddings! I also enjoy cooking – this is a stress-free way to express myself. Moreover, I love to travel – to learn about other cultures, histories, and beautiful scenery.

Part 2: Anthony Grace – Selected questions from the Proust Questionnaire[1]

What is your idea of perfect happiness?
To be content with my achievements and to share my joy with others. To know I have done my best and lived life to the fullest. And to be recognized as a good person and a good scientist.

What is your greatest fear?
Fear of failure; being able to provide funds for those in my lab to continue their careers and to be able to keep positively contributing to the field. Additionally, I do not want my ego to be out of control: it is essential to remain humble and know that good fortune has helped me a lot.

Which living person do you most admire?
Scientifically, Peter Kalivas has demonstrated the perfect balance of a brilliant and productive scientist, a friendly and supportive person, and someone who focuses on their family. As far as famous people, I think that may be Taylor Swift – not only a brilliant musician but someone who is soulful, kind, and very generous to those around her and the disadvantaged.

What is your greatest extravagance?
I love to cook and also love to travel; the best part of traveling is the ability to form lasting friendships with colleagues from different countries and get the chance to explore new places.

What are you most proud of?
I am very proud of my children and the incredible and thoughtful human beings they have become.

[1]In the late nineteenth century, various questionnaires were a popular diversion designed to discover new things about old friends. What is now known as the 35-question Proust Questionnaire became famous after Marcel Proust's answers to these questions were found and published posthumously. Proust answered the questions twice, at ages 14 and 20. Multiple other historical and contemporary figures have answered the Proust Questionnaire, such as Oscar Wilde, Karl Marx, Arthur Conan Doyle, Stéphane Mallarmé, Paul Cézanne, Martin Boucher, Hugh Jackman, David Bowie, and Zendaya. The Proust Questionnaire is often used to interview celebrities: the idea is that by answering these questions, an individual will reveal his or her true nature. We have condensed the Proust Questionnaire by reducing the number of questions and slightly rewording some. These curated questions provide insights into the individual's inner world, ranging from notions of happiness and fear to aspirations and inspirations.

What is your greatest regret?
Spending more time with my parents while they were still alive.

What is the quality you most admire in people?
Compassion and honesty.

What do you consider the most overrated virtue?
Achieving wealth – especially when it comes at the expense of others.

What is your favorite activity (physical or intellectual)?
I love to walk; it is healthy and gives me time to think and explore. And music – I am very passionate about listening to new music.

Where would you most like to live?
I love living in Pittsburgh – where I grew up, and it is the perfect mix of a small-town attitude with big-city facilities. I also love to be in Italy; it is part of my ancestry, and the people are so lovely, the history incredible, and the food amazing!

What is your most treasured possession?
It is challenging since possessions are such transient things: probably my home, where I raised my kids, and where I feel most safe and comfortable.

When and where were you happiest? And why were you so happy then?
Graduate school was a great time of discovery, personally and professionally, when I grew in so many dimensions.

What is your most marked characteristic?
Being able to talk to anyone and appreciate people for who they are rather than what they have.

Among your talents, which one gives you a competitive edge?
Easy – being passionate about my work and discovering things that can help others in their lives.

What is a personality/characteristic trait you wish you had?
Patience – I have some, but I need to lay back and reflect more.

What do you consider your greatest achievement?
The people who have come through my lab and the success they have had as independent investigators.

What do you most value in your friends?
Honesty and a great sense of humor.

Who are your favorite writers?
I love science fiction, so Isaac Asimov and Frank Herbert.

Who are your heroes of fiction?
I always liked Batman – because of his intellect and compassion for people.

Who are your heroes in real life?
I think that Keanu Reeves is a person I would emulate – he is humble and generous despite being through a rough life.

What aphorism or motto best encapsulates your life philosophy?
I like to trust everyone explicitly until they show me that they cannot be trusted. And be kind to everyone; you do not know what trauma they are carrying.

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INNOVATORS & IDEAS: RESEARCH LEADER

Consuelo Walss-Bass: Why does my sister have schizophrenia and I do not? Understanding how a person's unique genetic makeup interacts with their environment to shape behavior is one of the final frontiers in medicine

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In this Genomic Press Interview, Dr. Consuelo Walss-Bass, a groundbreaking researcher in psychiatric genetics, explores the fundamental question that has shaped her scientific journey: "Why does my sister have schizophrenia and I do not?" As the John S. Dunn Foundation Distinguished Chair in Psychiatry at the University of Texas Health Science Center at Houston, Dr. Walss-Bass has dedicated her career to unraveling the complex biological mechanisms underlying severe mental health disorders. Her groundbreaking work integrates genomic, proteomic, and clinical research to translate genetic discoveries into practical applications for patient care. Born and educated in Torreón, Mexico, Dr. Walss-Bass overcame significant barriers in engineering to become a distinguished scientist, establishing the UTHealth-Houston Brain Collection as an invaluable resource for studying the molecular foundations of mental illness. Her innovative work with induced pluripotent stem cells has opened new avenues for personalized psychiatric medicine, while her integrated approach to understanding how genetic makeup interacts with environmental factors represents one of the most promising frontiers in psychiatry. Beyond her research contributions, she demonstrates a profound commitment to mentoring the next generation of scientists, particularly those from underrepresented backgrounds. Through this comprehensive interview, Dr. Walss-Bass shares insights into both her personal connection to mental illness that motivated her transition from cancer research to psychiatric genetics and her vision for destigmatizing mental health disorders by uncovering their biological underpinnings, ultimately aiming to improve diagnosis, treatment, and public understanding of conditions like schizophrenia and bipolar disorder.

Figure 1. Consuelo (Chelo) Walss-Bass, MS, PhD, University of Texas Health Science Center at Houston, USA.

Part 1: Consuelo Walss-Bass – Life and Career

Could you give us a glimpse into your personal history, emphasizing the pivotal moments that first kindled your passion for science?

I discovered my love for science during middle school, when my chemistry professor introduced the atom; I could picture the electrons in their orbitals in my mind. I also loved biology and was amazed by how a microscopic cellular organism functioned intricately. I wanted to study biochemistry, the combination of chemistry and biology, but no biochemistry degree was offered in my hometown of Torreon, Mexico. My father did not have the money to send me to college elsewhere, and the fact that at that time, girls my age did not usually leave home in Mexico pushed me to pursue the closest thing available at the university in Torreon: Chemical Engineering. I actually liked engineering and thought I would get a job in industry after college, but I soon found a harsh reality: back then, no industrial company in Torreon would hire a woman engineer. So, I decided to go to the U.S. and pursue a Master's Degree in Chemistry. I was nervous about leaving my family, my friends, and my country, so I decided to go to the University of Texas in San Antonio because I had a close childhood friend who lived in San Antonio, and this was not too far from home. It was during my master's that I found out I loved doing research and working with my thesis mentor on nucleotide-metal complexes. I also rediscovered my passion for biochemistry. My next step was to pursue a Ph.D. in Biochemistry at San Antonio's Health Sciences Center. After all these years, it is still amazing that life allowed me to fulfill my childhood dreams of becoming a biochemist.

Please share with us what initially piqued your interest in your favorite research or professional focus area.

Until the end of my PhD, I did not have a plan for my future. I loved to learn new things, and I loved science, so it had been natural for me to continue studying as long as I could. For my dissertation, I worked on cancer-related

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Figure 2. The Walss Family, a source of strength and inspiration. From left to right: Mom, sister Jenny, Chelo, brother Leo, sister Paty, Dad.

research. It was not until I was about to finish that I knew I had to look for a postdoctoral fellowship and that this would likely be what I would do for the rest of my life. I decided to do psychiatric research. My mother has schizophrenia. I wanted to know: what caused this? Why did she behave the way she did? However, my mentors did not like my decision. "There is no basic science research going on in psychiatry", they said. "What are you going to do?" They thought that pursuing this path would be the end of my career. I looked up to my mentors, and I knew they had my best interest in mind, but I decided to follow my heart. This was one of the best decisions I have ever made. I joined the Department of Psychiatry as a postdoctoral fellow in 2001, when the human genome was about to be fully sequenced, and the search for causative genes in psychiatry was about to take off. It turned out I was in the right place at the right time. Because of my training in basic science research, I started collaborating with many clinicians in the department to correlate their patient's clinical measures with genetics, and I was soon promoted to Assistant Professor. This has been the basis of my work ever since, researching the interface between the bench and the bedside.

We would like to know more about your career trajectory leading up to your most relevant leadership role. What defining moments channeled you toward that leadership responsibility?

I believe a defining moment in my career was my decision to combine the psychiatric genetics training I received as a postdoctoral fellow with my graduate training in protein biochemistry and cellular biology to be able to investigate the functional relevance of genetic variants that were starting to be implicated in psychiatric disorders, and this allowed me to become an independent investigator.

What is a decision or choice that seemed like a mistake at the time but ended up being valuable or transformative for your career or life?

I took an 8 month break after finishing my PhD. I was initially concerned the break was too long and that it would be hard to come back to research. However, this turned out to be a very good decision because I was really burnt out at the time. Taking a break allowed me to refocus, get my energy back, and think about what I wanted to do next. It was during this break that I made the final decision to follow my heart and change the direction of my career from cancer to psychiatric genetics.

What habits and values did you develop during your academic studies or subsequent postdoctoral experiences that you uphold within your research environment?

My academic journey as a basic science researcher has taught me that a true scientist can pursue any area of research they want. Scientists should not put themselves in a box and should not be afraid of pursuing different areas of research. If they do not know how to do something, they can ask for help and collaborate with others who have expertise in that area. It is important not to be afraid to go where science leads. However, collaborating with others also means that one should not always insist on being the leader of a project or study. I have learned that being in the passenger seat is sometimes the best way to move forward and advance your own career.

Please tell us more about your current scholarly focal points within your chosen field of science.

Throughout my career I have used my diverse multidisciplinary training towards increasing the understanding of the biological underpinnings of mental health disorders. In addition to managing my research laboratory and all the work associated with it, I have had the honor of leading diverse programs. I highlight two examples here. 1) Upon my arrival at UTHealth Houston in 2014, in close collaboration with the Harris County Institute of Forensic Sciences, I established the UTHealth Houston Brain Collection, a state-of-the-science center for utilizing human postmortem brain, blood, and skin biopsies, to help study brain disorders. Brain tissue provides a crucial resource for understanding the biological causes of substance misuse and mental illness. We preserve high-quality tissue in combination with extensive clinical information to drive evidence-based research. This effort involves working closely with family members, medical examiners, toxicologists, and clinical psychologists to obtain detailed clinical and behavioral information that can be used to connect biological processes within defined brain circuitry with behavior and personality measures. The brain tissue, medical records, and clinical information are available to other investigators to perform research. 2) Because of my strong experience in working at the interface between basic and clinical sciences, I was recently named Director of the Biobehavioral Health Research Core at the Cizik Nursing Research Institute at UT Health Houston, charged with establishing a model of collaboration and dialogue between investigators working in the laboratory and nurses who are working directly with patients and are interested in doing research. In this role, I hope to help nurse scientists advance their research and become successful in obtaining funding to bridge biology with behavioral outcomes.

What impact do you hope to achieve in your field by focusing on specific research topics?

I am trained as a researcher and have a strong passion to work towards unraveling the mysteries underlying behavioral disorders. I want to find cures, I want to find better treatments, but I also want to identify what is causing these disorders so I can help explain this to people that are suffering from them, to families and to communities, and perhaps in this way demystify the experience of these illnesses for both patients and the public. I want to educate the community at large to reduce the stigma associated with these disorders, to help people see them as they do cancer or diabetes.

What do you most enjoy in your capacity as an academic or research leader?
What I enjoy the most is the opportunity to collaborate with scientists from all over the world. These collaborations provide a rich source of resources to advance my research and enrich my personal life because I have grown so much by learning from other people's backgrounds and experiences.

At Genomic Press, we prioritize fostering research endeavors based solely on their inherent merit, uninfluenced by geography or the researchers' personal or demographic traits. Are there particular cultural facets within the scientific community that warrant transformative scrutiny, or is there a cause within science that you feel strongly devoted to?
I am passionate about mentoring students and encouraging them to pursue their dreams. Many students think that science is too hard and that they will not be able to go far in this field. I believe that by having a role model, students can see that they can achieve anything they strive for. I am particularly focused on mentoring women and underrepresented minorities.

Outside professional confines, how do you prefer to allocate your leisure moments, or conversely, in what manner would you envision spending these moments given a choice?
I love to read. It takes me a while to finish a book because I sometimes read a page repeatedly, trying to imagine and live out what I am reading.

Part 2: Consuelo Walss-Bass – Selected questions from the Proust Questionnaire[1]

What is your most marked characteristic?
Loyalty.

Among your talents, which one(s) give(s) you a competitive edge?
I am very good at doing puzzles. I can see things from different angles and put them together.

If you could change one thing about yourself, what would it be?
I have trouble enjoying the moment, not thinking about what I must do next. I want to be able to turn this off and just enjoy the ride more.

What is your current state of mind?
At peace.

What is your idea of perfect happiness?
Enjoying a cup of hot coffee in the morning.

When and where were you happiest? And why were so happy then?
I am the happiest right now. I have two amazing sons, a loving and supportive husband, and a great career.

What is your greatest fear?
That one of my sons will have schizophrenia.

What is your greatest regret?
Not recognizing my sister Paty needed help years before she was diagnosed with schizophrenia.

What are you most proud of?
My children.

What do you consider your greatest achievement?
Helping my sister Paty to be stable.

What or who is your greatest passion?
Social justice. I believe we still have a long way to go to achieve true equality for all, and I want to do whatever I can to help in this cause.

What is your favorite occupation (or activity)?
Reading a good book.

What is your greatest extravagance?
A day at the spa.

What is your most treasured possession?
My mind.

Where would you most like to live?
Somewhere where the temperature is never too hot or too cold.

What is the quality you most admire in people?
Honesty.

What is the trait you most dislike in people?
Unwillingness to accept responsibility.

What do you consider the most overrated virtue?
None, we need to value all of them more.

What do you most value in your friends?
Getting an honest opinion from them, not what they think I want to hear.

Which living person do you most admire?
Pope Francis. His empathy.

Who are your heroes in real life?
My father. He taught me everything by saying very little.

If you could have dinner with any historical figure, who would it be and why?
Marie Curie. As a woman scientist she was a trailblazer, way ahead of her time.

Who are your favorite writers?
Charles Dickens and Alexandre Dumas.

Who are your heroes of fiction?
Superman. He was always ready to go to the rescue.

What aphorism or motto best encapsulates your life philosophy?
Do not leave for tomorrow what you can do today.

Houston, Texas, USA
10 March 2025

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[1] In the late nineteenth century, various questionnaires were a popular diversion designed to discover new things about old friends. What is now known as the 35-question Proust Questionnaire became famous after Marcel Proust's answers to these questions were found and published posthumously. Proust answered the questions twice, at ages 14 and 20. In 2003 Proust's handwritten answers were auctioned off for $130,000. Multiple other historical and contemporary figures have answered the Proust Questionnaire, including among others Karl Marx, Oscar Wilde, Arthur Conan Doyle, Fernando Pessoa, Stéphane Mallarmé, Paul Cézanne, Vladimir Nabokov, Kazuo Ishiguro, Catherine Deneuve, Sophia Loren, Gina Lollobrigida, Gloria Steinem, Pelé, Valentino, Yoko Ono, Elton John, Martin Scorsese, Pedro Almodóvar, Richard Branson, Jimmy Carter, David Chang, Spike Lee, Hugh Jackman, and Zendaya. The Proust Questionnaire is often used to interview celebrities: the idea is that by answering these questions, an individual will reveal his or her true nature. We have condensed the Proust Questionnaire by reducing the number of questions and slightly rewording some. These curated questions provide insights into the individual's inner world, ranging from notions of happiness and fear to aspirations and inspirations.

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VIEWPOINT

Recent findings and future directions for the intersection of genetic and environmental contributions to schizophrenia

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It is well established that both genetic and environmental factors contribute to risk for schizophrenia (SCZ), and much progress has been made in identifying the specific factors conferring risk. However, the nature and extent of interactions between them has long been a topic of debate. Both the data and methods available to address this have evolved rapidly, enabling new prospects for identifying gene–environment interactions in SCZ. To date, there is limited evidence of strong gene–environment interactions, with environmental factors, molecular genetic risk, and family history simultaneously contributing to risk of SCZ. Still, there are several enduring challenges, some of which can likely be addressed with new tools, methods, and approaches for investigating gene–environment interplay. Consequently, advancements in this field will enhance our capacity to identify individuals most vulnerable to specific environmental exposures, which is pivotal for targeted prevention and intervention.

Recent Findings from Molecular Genetics Studies

Family, twin, and adoption studies robustly support the role of genetic factors in schizophrenia (SCZ) (1–4). While early attempts to identify specific genetic markers through candidate gene studies faced challenges in reproducibility, these studies highlighted the importance of properly controlling for multiple testing to reduce the risk of false positives, as well as the need for large samples to detect variants with small effect sizes (5, 6). In the past 15 years, genome-wide association studies (GWAS) and the subsequent cascade of downstream analyses have made great strides in elucidating the genetic foundations of SCZ. Large-scale international collaborations have been instrumental in pooling resources, with the latest study amassing over 76,000 SCZ cases, and this has facilitated comprehensive investigation into the genetic basis of SCZ (7). It is now clear that SCZ is highly polygenic, with risk stemming from the cumulative influence of common and rare variants with small to moderate effect sizes (odds ratios 0.78–1.24) (7), and rare copy-number variants with strong effects (2 to >60x higher risk) (8, 9).

Concomitant with the emergence of genetic associations of high-confidence with SCZ, polygenic risk scores (PRS) were developed to quantify a person's predisposition for a disorder which is attributable to the additive impact of multiple common genetic variants (10). This risk is expressed as a single score, with single nucleotide polymorphisms (SNPs) weighted by their effect sizes from GWAS. SCZ-PRS offers a statistically significant but modest level of prediction and has been used to explore nosology and establish common genetic underpinnings with other psychiatric and somatic disorders (11). PRS methodologies are continually refined to enhance predictive power and improve performance across diverse populations (12, 13).

Established and Emerging Environmental Risk Factors

The majority of SCZ risk stems from genetic effects but 19%–36% of the risk arises from environmental sources (1, 2). Several environmental risk factors for SCZ have been consistently identified in large-scale epidemiological studies, including cannabis use, pregnancy and birth complications, infections, winter birth, migration, urban upbringing, stressful life events, and childhood adversity (14–17). Air pollution is an emerging risk factor (18) that is complex and typically entwined with social inequality, and there are likely other unexplored environmental and chemical-based risk factors awaiting discovery. While the prevalence of these environmental factors varies across populations, they often disproportionately affect more disadvantaged groups. Some of the identified risk factors are quite common, for instance, childhood adversity (which encompasses parental separation) and adverse perinatal factors each have a population prevalence of ~40% in modern western cohorts (19, 20). Despite the widespread occurrence of environmental risk factors, only a subset of exposed individuals develops SCZ, which strongly suggests differential sensitivity due to underlying genetic predisposition.

Investigations of environmental risk have predominantly involved pursuing individual risk factors in a hypothesis-driven manner, somewhat echoing the early genetic approaches. Just as genetic risk exerts effects through the cumulative impact of multiple genetic factors, it has been proposed that environmental risk may similarly arise from accumulated exposure to a range of adverse environmental factors (21). Over the life course, individuals are subjected to myriad interconnected environmental exposures at different developmental stages, each potentially having protective, neutral, or negative impacts on psychiatric risk. This concept, termed the "exposome," encompasses the entirety of environmental exposures from conception onward (21).

Mirroring PRS approaches, there have been attempts to generate an exposome score weighted by the effect sizes of the environmental factors for SCZ phenotypes (22–25). Unlike genetic studies, which typically require only a single blood sample to derive genetic risk, exposome research requires richly phenotyped, longitudinal, population-based cohorts. While this research is still in early stages, there is optimism that embracing the complexity and dynamic nature of environmental exposures will deliver further elucidation of their collective influence on SCZ.

Is Gene-Environment Interplay the Missing Link?

Exploring gene-environment interplay, which encompasses both gene-environment correlation (where genotype influences exposure to environmental factors, termed rGE) and gene–environment interaction (where the effect of the genotype depends on the presence of an environmental factor, or vice versa, termed G × E), holds promise for gaining further insight into the etiology of SCZ.

The SNP-based heritability of SCZ identified in GWAS accounts for ~24% of the variance, a stark contrast to the estimates of ~80% from twin studies (2, 4, 7). While rare genetic variation accounts for some of

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the discrepancy, G × E has been theorized to at least partially explain this heritability gap, and this is supported by one recent study (26).

Early G × E studies in SCZ relied on proxies such as family history for genetic risk assessment, or examined single candidate genes, as summarized by earlier reviews (17, 27, 28). These studies encountered similar power issues and biases as candidate gene association studies and often failed to replicate. Genome-wide approaches are considered superior to hypothesis-driven methods for genetic associations but require prohibitively large samples for G × E studies. Therefore, gene prioritization strategies are essential. In one successful example, a genome-wide environment interaction study used a two-stage design to reveal a significant interaction between *in utero* exposure to cytomegalovirus infection and a variant within the *CTNNA3* gene (29). First, the association between the exposure and the complete set of SNPs was assessed, then these prioritized SNPs were examined further to identify interaction effects for the outcome. This variant was not previously linked to SCZ, and this interaction was subsequently replicated (30).

In recent years, a few studies have investigated G × E interactions using PRS as an indicator of genetic liability to SCZ. Most of these studies report independent effects of PRS and environmental exposures and no evidence for multiplicative interactions, including for infections (31), adverse perinatal factors (32, 33), and childhood adversity (34). One study found evidence for an additive interaction effect between SCZ-PRS and childhood adversity on psychosis phenotypes—but it was mediated by a measure of affective dysregulation (35). Even for cannabis use, which demonstrates modest genetic correlations with SCZ (36–38), G × E studies report null interactions. Similarly, for urbanicity, studies support a degree of rGE (39, 40), but null interaction effects for birth in densely populated areas on SCZ risk (41). Still, large-scale genetic studies have rarely considered the impact of variation in environmental risk, highlighting the need for further research in this area.

On the other hand, positive additive interactions have been observed between dichotomized SCZ-PRS and certain environmental factors such as lifetime regular cannabis use and early-life adversities (42). These findings suggest a synergistic effect, indicating that the combined influence of genetic predisposition and environmental exposure exceeds the sum of their individual effects. There was no evidence of interaction effects for winter birth, hearing impairment, or child abuse. Positive additive interactions have also been identified for exposome risk scores and SCZ-PRS for SCZ spectrum disorders (24, 43, 44). Still, there is the need for confirmatory studies in large cohorts and different populations.

Presently, findings from PRS studies do not support the classic G × E (multiplicative) interaction model, whereby genotype and environmental factors only exert effects when both are present. Instead, current evidence suggests that genetic and environmental factors both contribute to risk through either independent or additive effects. However, statistical considerations for detecting and interpreting G × E interactions, such as choice of scale and model selection, are often overlooked. These issues have been extensively discussed, with recommendations for best practice (45, 46). Furthermore, it would be premature to entirely reject G × E hypotheses on the basis of PRS, which capture only a small portion of the expected genetic liability, among other methodological limitations (47, 48).

Future Focus

The extent to which there is interplay between genetic, familial, and environmental factors in the development of SCZ is still largely unknown. While we now possess a wealth of data on genetic and environmental risk factors, the challenge lies in making connections between them and then translating findings into clinically useful insights.

Challenges with GWAS and PRS Studies

Although findings from GWAS have provided useful biological insights into SCZ, they have yet to translate into tangible improvements in diagnosis and treatment. Despite their powerful impact on research, PRS have little clinical utility. Moreover, variations between the top and bottom percentiles might be exaggerated due to the case–control design of GWAS, with more modest risk prediction found in other real-world settings such as electronic health records (49). Assortative mating and rGE can also contribute to inflation of GWAS estimates (50). To address this, family-based GWAS designs have been utilized for several disorders by constructing PRS from non-transmitted parental alleles, albeit not yet implemented for SCZ (51). These designs can help identify rare variants and provide less biased estimates of direct genetic effects by reducing confounding from assortative mating and population stratification (51); however, they pose challenges in terms of recruitment of family members of individuals with SCZ, acquiring informed consent, and limited statistical power.

As GWAS sample sizes have increased, so has the proportion of the variance explained by PRS, nevertheless a ceiling effect is impending, whereby further increases in sample size will yield diminishing returns in explanatory power (52). However, these scores may have other useful applications, through correlations with symptoms and clinical features they may prove valuable in distinguishing between psychiatric disorders and optimal treatment approaches (53, 54).

Expanding the Analytical Toolkit

Although they minimize the multiple testing burden, PRS are likely too broad to be useful for more specific G × E interactions, necessitating more focused approaches and methodological tools. For instance, two-step designs which reduce the initial pool of target SNPs are a resourceful way to circumnavigate the prohibitive multiple testing burden (29, 55, 56). Fine-mapping methods reduce GWAS-derived loci to a smaller set of likely causal variants and can aid prioritization of genes for downstream G × E analyses (7). Modified PRS approaches endeavor to enhance polygenic risk prediction by leveraging correlated phenotypes (57), while others focus on enrichment of genetic variants at the biological pathway level (58).

Beyond genomics, various omics technologies have been applied to examine different aspects of SCZ pathogenesis and may yield further insights about the intermediary paths between genotype and environmental factors (59). These advancements offer novel avenues for capturing genetic risk and biomarkers for downstream application in gene-environment studies.

Other Sources of Genetic Variation

While recent focus has been on identifying common genetic variants associated with SCZ, rare genetic variants remain relatively unexplored in the context of G × E interactions. Only recently have large-scale collaborations, like the Schizophrenia Exome Sequencing Meta-Analysis (SCHEMA) consortium, amassed sufficient sequence data from many studies to identify rare genetic variants with exome-wide significance. The study identified ultra-rare coding variants in 10 genes with strong effect sizes (odds ratios 3–50, $P < 2.14 \times 10^{-6}$) and overlapping findings with the most recent GWAS (60). However, several rare copy-number variations (CNVs), involving deletions or duplications of large segments of DNA, have been identified which can have substantial impact on risk of SCZ. Individuals carrying associated CNVs, such as the 22q11.2 deletion, may be more likely to be exposed to adverse environmental exposures due to the impact on medical, social, and cognitive aspects (61). It has been reported that lifetime stress may influence psychosis risk symptoms in 22q11.2 deletion carriers, suggesting that it may be worth further investigating the role of environmental factors in the expression of psychosis risk among those with CNVs (62). Rare variants could be a promising avenue of exploration in a precision medicine context given that they are a single locus of strong effect, yet their rarity poses methodological challenges in terms of garnering adequate statistical power for scientific investigation. The scarcity of G × E studies using rare variants limits the field's current comprehension of the genetic component of the interaction.

The spotlight on molecular methodologies in human genetics should not overlook the significance of familial phenotypic records in genetic medicine and genetic epidemiology (63). There are several recent and emerging methods for model-based estimates of liability from family records, such as family genetic risk scores (FGRS) (64), the liability threshold on family history (LT-FH) (65), and Pearson-Aitken family genetic risk scores (PA-FGRS) (66). FGRS have already been used to evaluate

diagnostic stability, genetic architecture, and clinical features of several psychiatric disorders (67, 68). Although counterintuitive, PRS and indicators of family history have low correlations and appear to contribute independently to SCZ prediction (66).

Increasing Ancestral Diversity

The overwhelming majority of molecular genetic studies have been conducted in populations of European ancestry, potentially exacerbating health inequalities and impeding scientific progress (69). Several initiatives are underway to diversify these samples (7, 70), which will provide opportunities to increase our understanding of genetic risk across different environments, cultures, and ancestries.

As with the genetic findings, the bulk of the reliable evidence on environmental risk factors primarily stems from European and North American studies. Nordic registers, documenting numerous medical, social and demographic factors for the entire population from birth, are a rich resource for investigating the impact of environmental risk factors in rare psychiatric disorders and have provided some of the most robust epidemiological estimates (20, 71, 72).

Exploring more diverse settings and countries with greater environmental variability will likely clarify whether there are key cultural differences and aid understanding of true etiological associations. The challenge persists that to comprehensively investigate the genetic and environmental contributions to SCZ requires the rare combination of large, genotyped cohorts with longitudinal assessments of several environmental exposures over the life course.

Conclusion

There is still much to uncover regarding the interplay between genetic, familial, and environmental factors in SCZ. Undoubtedly, there are additional environmental factors and gene–environment interactions yet to be discovered. Given the high degree of shared genetic and environmental risk among psychiatric disorders, exploring G × E may help to isolate disorder-specific associations and pinpoint mediating or moderating biological pathways. Advancements in genetic and statistical tools will likely accelerate G × E research and maximize the utilization of existing datasets. The prospect of identifying individuals most vulnerable to specific environmental exposures underscores the importance of further exploration, offering opportunities for prevention and intervention.

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References

1. Lichtenstein P, Yip BH, Björk C, Pawitan Y, Cannon TD, Sullivan PF, et al. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet. 2009;373(9659):234–9. DOI: 10.1016/S0140-6736(09)60072-6. PMID: 19150704; PMCID: PMC3879718
2. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry. 2003;60(12):1187–92. DOI: 10.1001/archpsyc.60.12.1187. PMID: 14662550
3. Kendler KS. What psychiatric genetics has taught us about the nature of psychiatric illness and what is left to learn. Mol Psychiatry. 2013;18(10):1058–66. DOI: 10.1038/mp.2013.50. PMID: 23628988
4. Hilker R, Helenius D, Fagerlund B, Skytthe A, Christensen K, Werge TM, et al. Heritability of schizophrenia and schizophrenia spectrum based on the nationwide danish twin register. Biol Psychiatry. 2018;83(6):492–8. DOI: 10.1016/j.biopsych.2017.08.017. PMID: 28987712
5. Johnson EC, Border R, Melroy-Greif WE, de Leeuw CA, Ehringer MA, Keller MC. No evidence that schizophrenia candidate genes are more associated with schizophrenia than noncandidate genes. Biol Psychiatry. 2017;82(10):702–8. DOI: 10.1016/j.biopsych.2017.06.033. PMID: 28823710; PMCID: PMC5643230
6. Colhoun HM, McKeigue PM, Smith GD. Problems of reporting genetic associations with complex outcomes. Lancet. 2003;361(9360):865–72. DOI: 10.1016/s0140-6736(03)12715-8. PMID: 12642066
7. Trubetskoy V, Pardiñas AF, Qi T, Panagiotaropoulou G, Awasthi S, Bigdeli TB, et al. Mapping genomic loci implicates genes and synaptic biology in schizophrenia. Nature. 2022;604(7906):502–8. DOI: 10.1038/s41586-022-04434-5. PMID: 35396580; PMCID: PMC9392466
8. Rees E, Kirov G. Copy number variation and neuropsychiatric illness. Curr Opin Genet Dev. 2021;68:57–63. DOI: 10.1016/j.gde.2021.02.014. PMID: 33752146; PMCID: PMC8219524
9. Marshall CR, Howrigan DP, Merico D, Thiruvahindrapuram B, Wu W, Greer DS, et al. Contribution of copy number variants to schizophrenia from a genome-wide study of 41,321 subjects. Nat Genet. 2017;49(1):27–35. DOI: 10.1038/ng.3725. PMID: 27869829; PMCID: PMC5737772
10. International Schizophrenia Consortium, Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature. 2009;460(7256):748–52. DOI: 10.1038/nature08185. PMID: 19571811; PMCID: PMC3912837
11. Lewis CM, Vassos E. Polygenic scores in psychiatry: on the road from discovery to implementation. Am J Psychiatry. 2022;179(11):800–6. DOI: 10.1176/appi.ajp.20220795. PMID: 36317334
12. Kachuri L, Chatterjee N, Hirbo J, Schaid DJ, Martin I, Kullo IJ, et al. Principles and methods for transferring polygenic risk scores across global populations. Nat Rev Genet. 2024;25(1):8–25. DOI: 10.1038/s41576-023-00637-2. PMID: 37620596; PMCID: PMC10961971
13. Choi SW, Mak TS-H, O'Reilly PF. Tutorial: a guide to performing polygenic risk score analyses. Nat Protoc. 2020;15(9):2759–72. DOI: 10.1038/s41596-020-0353-1. PMID: 32709988; PMCID: PMC7612115
14. Vassos E, Agerbo E, Mors O, Pedersen CB. Urban–rural differences in incidence rates of psychiatric disorders in Denmark. Br J Psychiatry. 2016;208(5):435–40. DOI: 10.1192/bjp.bp.114.161091. PMID: 26678865
15. Brown AS. The environment and susceptibility to schizophrenia. Pro Neurobiol. 2011;93(1):23–58. DOI: 10.1016/j.pneurobio.2010.09.003. PMID: 20955757; PMCID: PMC3521525
16. Belbasis L, Köhler CA, Stefanis N, van Os J, Vieta E, Seeman MV, et al. Risk factors and peripheral biomarkers for schizophrenia spectrum disorders: an umbrella review of meta-analyses. Acta Psychiatr Scand. 2018;137(2):88–97. DOI: 10.1111/acps.12847. PMID: 29288491
17. Robinson N, Bergen SE. Environmental risk factors for schizophrenia and bipolar disorder and their relationship to genetic risk: current knowledge and future directions. Front Genet. 2021;12:686666. DOI: 10.3389/fgene.2021.686666. PMID: 34262598; PMCID: PMC8273311
18. Khan A, Plana-Ripoll O, Antonsen S, Brandt J, Geels C, Landecker H, et al. Environmental pollution is associated with increased risk of psychiatric disorders in the US and Denmark. PLoS Biol. 2019;17(8):e3000353. DOI: 10.1371/journal.pbio.3000353. PMID: 31430271; PMCID: PMC6701746
19. Robinson N, Ploner A, Leone M, Lichtenstein P, Kendler KS, Bergen SE. Impact of early-life factors on risk for schizophrenia and bipolar disorder. Schiz Bull. 2023;49(3):768–77. DOI: 10.1093/schbul/sbac205. PMID: 36946500; PMCID: PMC10154720
20. Robinson N, Ploner A, Leone M, Lichtenstein P, Kendler KS, Bergen SE. Environmental risk factors for schizophrenia and bipolar disorder from childhood to diagnosis: a swedish nested case-control study. Psychol Med. 2024:1–10. DOI: 10.1017/S0033291724000266. PMID: 38425272
21. Erzin G, Gülöksüz S. The exposome paradigm to understand the environmental origins of mental disorders. Alpha Psychiatry. 2021;22(4):171–6. DOI: 10.5152/alphapsychiatry.2021.21307. PMID: 36424935; PMCID: PMC9590645
22. Padmanabhan JL, Shah JL, Tandon N, Keshavan MS. The "polyenviromic risk score": aggregating environmental risk factors predicts conversion to psychosis in familial high-risk subjects. Schizophr Res. 2017;181:17–22. DOI: 10.1016/j.schres.2016.10.014. PMID: 28029515; PMCID: PMC5365360
23. Vassos E, Sham P, Kempton M, Trotta A, Stilo SA, Gayer-Anderson C, et al. The Maudsley environmental risk score for psychosis. Psychol Med. 2020;50(13):2213–20. DOI: 10.1017/S0033291719002319. PMID: 31535606; PMCID: PMC7557157
24. Mas S, Boloc D, Rodríguez N, Mezquida G, Amoretti S, Cuesta MJ, et al. Examining gene–environment interactions using aggregate scores in a first-episode psychosis cohort. Schizophr Bull. 2020;46(4):1019–25. DOI: 10.1093/schbul/sbaa012. PMID: 32083289; PMCID: PMC7342095
25. Pries LK, Lage-Castellanos A, Delespaul P, Kenis G, Luykx JJ, Lin BD, et al. Estimating exposome score for schizophrenia using predictive modeling approach in two independent samples: the results from the EUGEI study. Schizophr Bull. 2019;45(5):960–5. DOI: 10.1093/schbul/sbz054. PMID: 31508804; PMCID: PMC6737483
26. Zhang H, Khan A, Kushner SA, Rzhetsky A. Dissecting schizophrenia phenotypic variation: the contribution of genetic variation, environmental exposures, and gene–environment interactions. Schizophrenia (Heidelb). 2022;8(1):51. DOI: 10.1038/s41537-022-00257-5. PMID: 35853906; PMCID: PMC9261082
27. Assary E, Vincent JP, Keers R, Pluess M. Gene-environment interaction and psychiatric disorders: review and future directions. Semin Cell Dev Biol. 2018;77:133–43. DOI: 10.1016/j.semcdb.2017.10.016. PMID: 29051054
28. Van Os J, Rutten BP, Poulton R. Gene–environment interactions in schizophrenia: review of epidemiological findings and future directions. Schizophr Bull. 2008;34(6):1066–82. DOI: 10.1093/schbul/sbn117. PMID: 18791076; PMCID: PMC2632485

29. Børglum A, Demontis D, Grove J, Pallesen J, Hollegaard MV, Pedersen CB, et al. Genome-wide study of association and interaction with maternal cytomegalovirus infection suggests new schizophrenia loci. Mol Psychiatry. 2014;19(3):325–33. DOI: 10.1038/mp.2013.2. PMID: 23358160; PMCID: PMC3932405
30. Avramopoulos D, Pearce BD, McGrath J, Wolyniec P, Wang R, Eckart N, et al. Infection and inflammation in schizophrenia and bipolar disorder: a genome wide study for interactions with genetic variation. PLoS One. 2015;10(3):e0116696. DOI: 10.1371/journal.pone.0116696. PMID: 25781172; PMCID: PMC4363491
31. Benros ME, Trabjerg BB, Meier S, Mattheisen M, Mortensen PB, Mors O, et al. Influence of polygenic risk scores on the association between infections and schizophrenia. Biol Psychiatry. 2016;80(8):609–16. DOI: 10.1016/j.biopsych.2016.04.008. PMID: 27364036
32. Valli I, Gonzalez Segura A, Verdolini N, Garcia-Rizo C, Berge D, Baeza I, et al. Obstetric complications and genetic risk for schizophrenia: differential role of antenatal and perinatal events in first episode psychosis. Acta Psychiatr Scand. 2023;148(1):81–90. DOI: 10.1111/acps.13546. PMID: 36912272
33. Vassos E, Kou J, Tosato S, Maxwell J, Dennison CA, Legge SE, et al. Lack of support for the genes by early environment interaction hypothesis in the pathogenesis of schizophrenia. Schizophr Bull. 2022;48(1):20–6. DOI: 10.1093/schbul/sbab052. PMID: 33987677; PMCID: PMC8781344
34. Trotta A, Iyegbe C, Di Forti M, Sham PC, Campbell DD, Cherny SS, et al. Interplay between schizophrenia polygenic risk score and childhood adversity in first-presentation psychotic disorder: a pilot study. PLoS One. 2016;11(9):e0163319. DOI: 10.1371/journal.pone.0163319. PMID: 27648571; PMCID: PMC5029892
35. Van Os J, Pries L-K, Ten Have M, de Graaf R, van Dorsselaer S, Delespaul P, et al. Evidence, and replication thereof, that molecular-genetic and environmental risks for psychosis impact through an affective pathway. Psychol Med. 2022;52(10):1910–22. DOI: 10.1017/S0033291720003748. PMID: 33070791
36. Pasman JA, Verweij KJH, Gerring Z, Stringer S, Sanchez-Roige S, Treur JL, et al. GWAS of lifetime cannabis use reveals new risk loci, genetic overlap with psychiatric traits, and a causal effect of schizophrenia liability. Nat Neurosci. 2018;21(9):1161–70. DOI: 10.1038/s41593-018-0206-1. PMID: 30150663; PMCID: PMC6386176
37. Johnson EC, Colbert SMC, Jeffries PW, Tillman R, Bigdeli TB, Karcher NR, et al. Associations between cannabis use, polygenic liability for schizophrenia, and cannabis-related experiences in a sample of cannabis users. Schizophr Bull. 2023;49(3):778–87. DOI: 10.1093/schbul/sbac196. PMID: 36545904; PMCID: PMC10154717
38. Cheng W, Parker N, Karadag N, Koch E, Hindley G, Icick R, et al. The relationship between cannabis use, schizophrenia, and bipolar disorder: a genetically informed study. Lancet Psychiatry. 2023;10(6):441–51. DOI: 10.1016/S2215-0366(23)00143-8. PMID: 37208114; PMCID: PMC10311008
39. Maxwell JM, Coleman JRI, Breen G, Vassos E. Association between genetic risk for psychiatric disorders and the probability of living in urban settings. JAMA Psychiatry. 2021;78(12):1355–64. DOI: 10.1001/jamapsychiatry.2021.2983. PMID: 34705035; PMCID: PMC8552117
40. Sariaslan A, Fazel S, D'onofrio BM, Långström N, Larsson H, Bergen SE, et al. Schizophrenia and subsequent neighborhood deprivation: revisiting the social drift hypothesis using population, twin and molecular genetic data. Transl Psychiatry. 2016;6(5):e796–6. DOI: 10.1038/tp.2016.62. PMID: 27138795; PMCID: PMC5070045
41. Paksarian D, Trabjerg BB, Merikangas KR, Mors O, Børglum AD, McGrath JJ, et al. The role of genetic liability in the association of urbanicity at birth and during upbringing with schizophrenia in Denmark. Psychol Med. 2018;48(2):305–14. DOI: 10.1017/S0033291717001696. PMID: 28659227; PMCID: PMC6361630
42. Guloksuz S, Pries L-K, Delespaul P, Kenis G, Luykx JJ, Lin BD, et al. Examining the independent and joint effects of molecular genetic liability and environmental exposures in schizophrenia: results from the EUGEI study. World Psychiatry. 2019;18(2):173–82. DOI: 10.1002/wps.20629. PMID: 31059627; PMCID: PMC6502485
43. Cuesta MJ, Papiol S, Ibañez B, García de Jalón E, Sánchez-Torres AM, Gil-Berrozpe GJ, et al. Effect of polygenic risk score, family load of schizophrenia and exposome risk score, and their interactions, on the long-term outcome of first-episode psychosis. Psychol Med. 2023;53(14):6838–47.
44. Pries LK, Dal Ferro GA, van Os J, Delespaul P, Kenis G, Lin BD, et al. Examining the independent and joint effects of genomic and exposomic liabilities for schizophrenia across the psychosis spectrum. Epidemiol Psychiatr Sci. 2020;29:e182. DOI: 10.1017/S2045796020000943. PMID: 33200977; PMCID: PMC7681168
45. Kendler KS, Gardner CO. Interpretation of interactions: guide for the perplexed. British J Psychiatry. 2010;197(3):170–1. DOI: 10.1192/bjp.bp.110.081331. PMID: 20807958
46. Roisman GI, Newman DA, Fraley RC, Haltigan JD, Groh AM, Haydon KC. Distinguishing differential susceptibility from diathesis–stress: recommendations for evaluating interaction effects. Dev Psychopathol. 2012;24(2):389–409. DOI: 10.1017/S0954579412000065. PMID: 22559121
47. Pingault JB, Allegrini AG, Odigie T, Frach L, Baldwin JR, Rijsdijk F, et al. Research review: how to interpret associations between polygenic scores, environmental risks, and phenotypes. J Child Psychology Psychiatry. 2022;63(10):1125–39. DOI: 10.1111/jcpp.13607. PMID: 35347715; PMCID: PMC9790749
48. Iyegbe C, Campbell D, Butler A, Ajnakina O, Sham P. The emerging molecular architecture of schizophrenia, polygenic risk scores and the clinical implications for GxE research. Soc Psychiatry Psychiatr Epidemiol. 2014;49(2):169–82. DOI: 10.1007/s00127-014-0823-2. PMID: 24435092
49. Zheutlin AB, Dennis J, Karlsson Linnér R, Moscati A, Restrepo N, Straub P, et al. Penetrance and pleiotropy of polygenic risk scores for schizophrenia in 106,160 patients across four health care systems. Am J Psychiatry. 2019;176(10):846–55. DOI: 10.1176/appi.ajp.2019.18091085. PMID: 31416338; PMCID: PMC6961974
50. Abdellaoui A, Yengo L, Verweij KJH, Visscher PM. 15 years of GWAS discovery: realizing the promise. Am J Hum Genet. 2023;110(2):179–94. DOI: 10.1016/j.ajhg.2022.12.011. PMID: 36634672; PMCID: PMC9943775
51. Howe LJ, Nivard MG, Morris TT, Hansen AF, Rasheed H, Cho Y, et al. Within-sibship genome-wide association analyses decrease bias in estimates of direct genetic effects. Nat Genet. 2022;54(5):581–92. DOI: 10.1038/s41588-022-01062-7. PMID: 35534559; PMCID: PMC9110300
52. Murray GK, Lin T, Austin J, McGrath JJ, Hickie IB, Wray NR. Could polygenic risk scores be useful in psychiatry?: a review. JAMA Psychiatry. 2021;78(2):210–9. DOI: 10.1001/jamapsychiatry.2020.3042. PMID: 33052393
53. Ikeda M, Saito T, Kanazawa T, Iwata N. Polygenic risk score as clinical utility in psychiatry: a clinical viewpoint. J Hum Genet. 2021;66(1):53–60. DOI: 10.1038/s10038-020-0814-y. PMID: 32770057
54. Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium; Ruderfer DM, Ripke S, McQuillin A, Boocock J, Stahl EA, et al. Genomic dissection of bipolar disorder and schizophrenia, including 28 subphenotypes. Cell. 2018;173(7):1705–1715.e16. DOI: 10.1016/j.cell.2018.05.046. PMID: 29906448; PMCID: PMC6432650
55. Murcray CE, Lewinger JP, Gauderman WJ. Gene-environment interaction in genome-wide association studies. Am J Epidemiol. 2009;169(2):219–26. DOI: 10.1093/aje/kwn353. PMID: 19022827; PMCID: PMC2732981
56. Gauderman WJ, Zhang P, Morrison JL, Lewinger JP. Finding novel genes by testing G × E interactions in a genome-wide association study. Genet Epidemiol. 2013;37(6):603–13. DOI: 10.1002/gepi.21748. PMID: 23873611; PMCID: PMC4348012
57. Zhang Q, Privé F, Vilhjálmsson B, Speed D. Improved genetic prediction of complex traits from individual-level data or summary statistics. Nat Commun. 2021;12(1):4192. DOI: 10.1038/s41467-021-24485-y. PMID: 34234142; PMCID: PMC8263809
58. Choi SW, García-González J, Ruan Y, Wu HM, Porras C, Johnson J, et al. PRSet: pathway-based polygenic risk score analyses and software. PLoS Genet. 2023;19(2):e1010624. DOI: 10.1371/journal.pgen.1010624. PMID: 36749789; PMCID: PMC9937466
59. Guan F, Ni T, Zhu W, Williams LK, Cui LB, Li M, et al. Integrative omics of schizophrenia: from genetic determinants to clinical classification and risk prediction. Mol Psychiatry. 2022;27(1):113–26. DOI: 10.1038/s41380-021-01201-2. PMID: 34193973; PMCID: PMC11018294
60. Singh T, Poterba T, Curtis D, Akil H, Al Eissa M, Barchas JD, et al. Rare coding variants in ten genes confer substantial risk for schizophrenia. Nature. 2022;604(7906):509–16. DOI: 10.1038/s41586-022-04556-w. PMID: 35396579; PMCID: PMC9805802
61. Beaton EA, Simon TJ. How might stress contribute to increased risk for schizophrenia in children with chromosome 22q11.2 deletion syndrome? J Neurodev Disord. 2011;3(1):68–75. DOI: 10.1007/s11689-010-9069-9. PMID: 21475728; PMCID: PMC3056992
62. Modasi J, Khachadourian V, O'Hora K, Kushan L, Slavich GM, Shields GS, et al. Associations between acute and chronic lifetime stressors and psychosis-risk symptoms in individuals with 22q11.2 copy number variants. Psychol Med. 2023;53(15):7222–31. DOI: 10.1017/S0033291723000740. PMID: 37078394; PMCID: PMC10719673
63. Kendler KS, Neale MC. "Familiality" or heritability. Arch Gen Psychiatry. 2009;66(4):452–3. DOI: 10.1001/archgenpsychiatry.2009.14. PMID: 19349315; PMCID: PMC4028600
64. Kendler KS, Ohlsson H, Sundquist J, Sundquist K. The patterns of family genetic risk scores for eleven major psychiatric and substance use disorders in a Swedish national sample. Transl Psychiatry. 2021;11(1):326. DOI: 10.1038/s41398-021-01454-z. PMID: 34045441 PMCID: PMC8160183
65. Hujoel MLA, Gazal S, Loh P-R, Patterson N, Price AL. Liability threshold modeling of case–control status and family history of disease increases association power. Nat Genet. 2020;52(5):541–7. DOI: 10.1038/s41588-020-0613-6. PMID: 32313248; PMCID: PMC7210076
66. Krebs MD, Hellberg K-LG, Lundberg M, Appadurai V, Ohlsson H, Pedersen E, et al. PA-FGRS is a novel estimator of pedigree-based genetic liability that complements genotype-based inferences into the genetic architecture of major depressive disorder. medRxiv. 2023. DOI: 10.1101/2023.06.23.23291611
67. Kendler KS, Ohlsson H, Sundquist J, Sundquist K. Family genetic risk scores and the genetic architecture of major affective and psychotic disorders in a Swedish national sample. JAMA Psychiatry. 2021;78(7):735–43. DOI: 10.1001/jamapsychiatry.2021.0336. PMID: 33881469; PMCID: PMC8060884
68. Kendler KS, Ohlsson H, Sundquist J, Sundquist K. Relationship of family genetic risk score with diagnostic trajectory in a swedish national sample of incident cases of major depression, bipolar disorder, other nonaffective psychosis, and schizophrenia. JAMA Psychiatry. 2023;80(3):241–9. DOI: 10.1001/jamapsychiatry.2022.4676. PMID: 36696095; PMCID: PMC9878431
69. Peterson RE, Kuchenbaecker K, Walters RK, Chen CY, Popejoy AB, Periyasamy S, et al. Genome-wide association studies in ancestrally diverse populations: opportunities, methods, pitfalls, and recommendations. Cell. 2019;179(3):589–603. DOI: 10.1016/j.cell.2019.08.051. PMID: 31607513; PMCID: PMC6939869
70. Lam M, Chen C-Y, Li Z, Martin AR, Bryois J, Ma X, et al. Comparative genetic architectures of schizophrenia in East Asian and European populations. Nat Genet. 2019;51(12):1670–8. DOI: 10.1038/s41588-019-0512-x. PMID: 31740837; PMCID: PMC6885121
71. Miettunen J, Suvisaari J, Haukka J, Isohanni M. Use of register data for psychiatric epidemiology in the Nordic countries. In Tsuang MT, Tohen M, Jones PB, eds. Textbook of Psychiatric Epidemiology. Chichester, UK: John Wiley & Sons Ltd; 2011;117–31. DOI: 10.1002/9780470976739.ch8
72. Bergen SE, Sullivan PF. National-scale precision medicine for psychiatric disorders in Sweden. Am J Med Genet B Neuropsychiatr Genet. 2018;177(7):630–4. DOI: 10.1002/ajmg.b.32562. PMID: 28686353

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PERSPECTIVE

Indigenous data protection in wastewater surveillance: balancing public health monitoring with privacy rights

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Wastewater-based epidemiology (WBE) has revolutionized public health surveillance by enabling real-time monitoring of disease patterns across populations through analysis of community wastewater. This innovative approach provides precise geographical tracking of pathogen levels and disease spread by detecting viral RNA and bacterial DNA signatures. Beyond pathogen detection, wastewater analysis reveals comprehensive community health data, including human genomic information and biomarkers of prescription medication and substance use patterns. For Indigenous populations, whose communities often occupy distinct geographical areas, this detailed biological data collection raises significant privacy and ethical concerns, particularly given historical patterns of research exploitation. By examining international case studies, we analyze instances where Indigenous genomic data and traditional knowledge have been misused in psychiatric and neuroscience research contexts, highlighting violations of informed consent principles, data sovereignty rights, and reinforcement of harmful stereotypes. The current regulatory gap in wastewater surveillance ethics necessitates the development of specialized WBE protocols for Indigenous communities. These guidelines must balance public health benefits with stringent privacy protections through authentic community engagement and Indigenous data governance rights recognition. This framework supports both epidemiological research advancement and the protection of Indigenous communities' autonomy in the age of genomic surveillance.

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Introduction

It is widely accepted that genetic factors contribute to the risk of developing a neuropsychiatric or neurodevelopmental disorder such as depression, schizophrenia, autism spectrum disorders, and addiction, among others (1–4). In 2023, for instance, a large multivariate genome-wide association meta-analysis showed 19 independent single-nucleotide polymorphisms (SNPs) significantly associated with general addiction risk and 47 SNPs for select substance disorders in individuals of European ancestry (5). In those of African descent, however, only a single SNP was associated with general addiction risk and another for risk of alcohol use disorder.

This study has a couple of noteworthy points. First, the authors were careful to point out that, despite interesting associations between polygenic risk scores and substance use disorders, their findings are not prognostic of future disease risk. This is an essential statement as past genomic studies have been used to promote the idea of genetic determinism, leading to racism and stereotyping (6, 7). For example, there has been a (not so) historical belief that alcoholism in Indigenous peoples was biological and that substance use and dependence in these communities was heritable (8), without any consideration of the transgenerational impacts of colonization and the colonial power structures that exist to this day. The second point of note is that the sample size for those of European descent (>1 million) was over 10 times higher than that of African descent, and those of Indigenous descent were not included in the study. This highlights the lack of participation of those from marginalized communities, and especially Indigenous communities, in genomic studies (5). There are reasons for this, a predominant one being a lack of transparency in many genomics research investigations that has led to a long-lasting mistrust of both research and researchers.

Wastewater-based surveillance and epidemiology (WBE) is an innovative public health approach that analyzes biological and chemical markers in wastewater to monitor the health of communities. Initially developed to assess trends in illicit drug use, WBE has expanded to include infectious disease surveillance, antimicrobial resistance tracking, and environmental monitoring. The COVID-19 pandemic highlighted the utility of WBE, as it served as an early warning system for outbreaks and a valuable tool for tracking viral variants in communities (9, 10). WBE provides critical insights into public health by enabling early detection of disease outbreaks through analyzing nucleic acid and other biomarkers in wastewater. This capability allows health authorities to implement timely interventions, potentially mitigating the spread of infections (11, 12). Beyond infectious disease monitoring, WBE assesses the presence of environmental pollutants, such as pesticides and pharmaceuticals, offering a holistic view of anthropogenic impacts on ecosystems (13).

DNA degradation in wastewater is influenced by a combination of chemical, biological, and environmental factors, including temperature, pH, microbial activity, and exposure to ultraviolet light. While wastewater treatment processes are designed to degrade organic materials, studies have shown that extracellular DNA can persist through various stages of treatment, raising concerns about the potential for recovering sensitive genetic information. Additionally, if nucleic acids are extracted from wastewater and stored in purified form at −80°C, they can remain intact indefinitely. For instance, Farkas *et al*. (14) demonstrated that while extensive decay of viral nucleic acids was observed during the storage of raw unprocessed wastewater, purified nucleic acid extracts stored at −80°C for 8–24 months showed little signs of degradation. Additionally, Acharya *et al*. (15) observed that specific bacterial DNA sequences exhibit resistance to degradation, especially in disinfected systems. This persistence has significant privacy implications, particularly in the context of WBE, where the unintended capture of human genomic material could threaten individual privacy if data are not adequately anonymized (16).

WBE holds significant potential for public health, but it also carries the risk of misuse, particularly in closed or marginalized communities such as Indigenous communities. Due to the substantial amount of human DNA in wastewater, detailed genomic analysis can reveal sensitive information about the genetic makeup, ancestry, health predispositions, and disease prevalence within a population. Those data could be used in

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closed communities to reinforce stereotypes, stigmatize groups, or exert control through targeted policies that disproportionately affect these populations. For example, genetic data could be linked to the prevalence of certain conditions to justify discriminatory health care practices or policies that ignore social determinants of health. Further, the small population sizes in these communities increase the risk of re-identification, breaching individual privacy despite aggregate-level analyses. Without stringent ethical guidelines, such practices could erode trust, infringe on autonomy, and exacerbate historical inequities faced by Indigenous peoples (17, 18). Ethical governance is critical to ensure that WBE serves public health purposes without exploiting or marginalizing vulnerable populations.

Building on these concerns, the potential misuse of wastewater surveillance to identify psychiatric disorders or drug use in marginalized populations raises additional ethical challenges. Psychiatric conditions are often stigmatized; therefore, associating these disorders with specific communities through wastewater analysis risks reinforcing harmful stereotypes and exacerbating social marginalization. This is particularly concerning in Indigenous communities, where historical trauma and systemic discrimination have already contributed to disproportionate mental health burdens (19). The ability to infer prevalence rates of psychiatric medication use, illicit drug use, or genetic markers associated with mental health conditions from wastewater data could inadvertently or intentionally be used to justify punitive or discriminatory interventions rather than addressing root causes such as poverty or inadequate access to healthcare. Furthermore, such data might be weaponized in policy debates, framing psychiatric conditions as cultural deficits rather than addressing structural inequalities (6, 20, 21). This underscores the urgent need for stringent data governance frameworks and community involvement in decision-making processes to prevent the misuse of surveillance technologies and respect these populations' autonomy and rights.

Mistakes of the Past: Genomics and Genetics Research in Indigenous Communities

Indigenous peoples are of great value in genomics research in large part due to their isolated genetic history. Whereas some researchers are interested in population genetics or genetic ancestry, that is, what the patterns of genetic variation can reveal about a community's history, societal structure, migration patterns, etc., others are interested in genetic information that may be medically (and commercially) valuable. Despite this, Indigenous communities are substantially underrepresented in genomics and genetics research, with many refusing to take part in these types of studies due to past exploitation. Examples include the unauthorized use, sharing, and patenting of genetic data, the lack of informed consent, cultural insensitivity, and the perpetuation of racial stereotypes (22–27).

One of the first recorded negative interactions between geneticists and an Indigenous community involved the Nuu-chah-nulth First Nations of Canada's Vancouver Island in British Columbia and a geneticist at the University of British Columbia (UBC) (27). In the early 1980s, the Nuu-chah-nulth provided blood samples for research into genetic causes of their high rates of rheumatoid arthritis. The researcher was unable to discover a genetic linkage to the disease; however, when they left UBC, they took the Nuu-chah-nulth samples with them to use in genomic ancestry research without the knowledge or consent of the tribe. Genomic ancestry research is a serious concern for many Indigenous peoples due to a disconnect between Indigenous origin stories and research findings. Notably, the DNA was not returned to the Nuu-chah-nulth until 20 years after sampling.

Perhaps one of the most egregious examples involving psychiatric research involves the Havasupai, a Native American tribe who live at the bottom of the Grand Canyon in Arizona in the United States (26, 28–30). In the early 1990s, community members approached researchers at Arizona State University (ASU) to initiate genetic research into diabetes as they were concerned about the increasing prevalence of the disease in their community. The Havasupai were told that the focus of the study would be on diabetes, but the project was designed to also focus on identifying genetic markers for schizophrenia. Researchers then generated a broad consent form to study "behavioral/medical disorders" signed by participating community members when the blood samples were taken. The ASU researchers were unable to find a genetic link to diabetes. Still, samples continued to be used for other research without specific consent, including studies on alcoholism, population migration, and inbreeding. They also continued the study into schizophrenia, which included unauthorized access to medical records. The Havasupai discovered the unauthorized use of their samples when a community member attended a seminar at ASU in 2003. In response, the tribe initiated legal action against ASU, which was resolved in a settlement in 2010. After the case, the Havasupai banned ASU researchers from entering their lands and conducting any form of research, following in the steps of the Navajo Nation, which passed a moratorium on genomic research within its boundaries in 2002.

At about the same time as the Havasupai study was being conducted, the Human Genome Diversity Project (HGDP) (31), the first large-scale genomics study, was initiated to explore global human genetic diversity by taking samples from isolated Indigenous populations (32). Some Indigenous communities had concerns over biopiracy, exploitation for profit, or that there would be access to the samples by an unknown number of researchers with various scientific goals (33). These concerns were not without merit (22, 33). Some participants were told their blood was being taken for pathology tests and then given to the HGDP. Cultural values in handling the samples were also not considered, and commercialization potential was not disclosed. In addition, informed consent was not always obtained. The enmity of Indigenous communities for the HGDP was only heightened when an anthropologist involved with the project was found to be also linked to the controversial collection of another set of samples collected from the Hagahai, an Indigenous people of central Papua New Guinea. The controversy surrounded a patent filed in 1990 by the National Institutes of Health (NIH) for a cell line derived from a Hagahai donor to commercialize his DNA for commercial profit (23). Shortly after the HGDP's failure, in 2005, another large-scale project, the Genographic Project, was launched to trace the migratory history of the human species through DNA. Like its HGDP predecessor, Indigenous communities also denounced it due to a lack of engagement and transparency (34). Sampling from Indigenous communities was prioritized as there was concern surrounding the mixing of the populations (34), thus building upon the old myth of the "Vanishing Indian." It was postulated that any subsequent findings of the HGDP and Genographic Project could lead to the genetic appropriation of culture, challenging cultural narratives about a people's origins and altering a group's understanding of themselves as a people (35).

In another example, geneticists from the Institute for Environmental Science and Research in New Zealand obtained samples from the Māori people with the intent of analyzing the monoamine oxidase-A (*MAOA*) gene as a marker for alcohol and tobacco dependence (25, 36, 37), as the enzyme MAO-A is involved in the breakdown of neurotransmitters such as dopamine and serotonin. In 2006, during the 11th International Congress of Human Genetics in Brisbane, Australia, the researchers announced that they had identified a genetic polymorphism in *MAOA* that was associated with low enzyme activity and higher dopamine levels in over half of the samples obtained from Māori men ($n = 17$) (25, 36, 37). A controversy ensued when one of the researchers mentioned the "warrior gene," a nickname given to the *MAOA* gene due to its reported link to aggression and criminal behavior (38, 39) in a media interview, also inferring that these attributes exist in the Māori people (25, 36, 37). In this example, the scientists drastically misstepped, not only by providing a harmful narrative unsupported by their findings to perpetuate racial stereotypes but also by generalizing to the entire Māori population.

The final example highlights the failure of research ethics boards (REBs) to ensure that adequate protocols were in place to protect Indigenous peoples. The Indigenous San peoples of South Africa are among the most sought-after Indigenous groups for population genomics research. In this case, the genomics research aimed to examine the genetic structure of four Indigenous Namibian "hunter-gatherers" and to compare their findings with that of a "Bantu from southern Africa." The findings were published in 2010 (40) and, along with its supplementary material, included conclusions and details (e.g., the terminology used) that the San regarded as private, pejorative, discriminatory, and inappropriate

(41). The San leadership was not consulted prior to publication, and their requests to the authors for information on the informed consent process were denied on the grounds that several REBs had approved the research. This interaction resulted in the development and publication of the San Code of Ethics (42) by the leaders of three San groups: the !Xun, Khwe, and !Khomani, a code that emphasizes respect, honesty, justice, fairness, care, and process.

The historical exploitation of Indigenous communities in genomics research offers critical lessons for contemporary challenges in WBE, particularly regarding ethical considerations such as informed consent, data ownership, community engagement, and the potential for stigmatization. The examples of the Havasupai Tribe (26, 28–30) and Nuu-chah-nulth First Nations (27) underscore the importance of transparency and highlight the consequences of neglecting autonomy and informed consent, such as long-term mistrust in scientific practices. Although data are collected at the community level rather than from individuals in WBE, concerns about privacy and consent persist, particularly when surveillance may expose sensitive health information without explicit community approval (43). The backlash against the Genographic Project by Indigenous groups (34) due to inadequate community engagement reflects the importance of involving affected populations in decision-making processes to foster trust—a principle equally applicable to public health surveillance initiatives. Moreover, data ownership and governance issues, as highlighted by the San Peoples' experience with genomics research (41), stress the necessity of clear data control frameworks in WBE to prevent misuse and ensure that communities benefit from the data collected. The harmful stereotyping seen in the Māori "warrior gene" controversy warns against using scientific findings to perpetuate negative narratives—a risk in WBE if data is poorly contextualized, potentially stigmatizing communities linked to specific health outcomes (25, 36, 37). Finally, the failure of REBs to adequately protect Indigenous interests in past genomics studies emphasizes the need for robust ethical oversight in public health surveillance to proactively address legal and privacy concerns (44). By applying these historical lessons, wastewater surveillance programs can balance public health benefits with respect for individual rights, data sovereignty, and cultural sensitivities, ultimately fostering greater public trust and ethical integrity.

A Call for the Development of an Ethical Indigenous Research Policy for WBE

The provided historical examples highlight past injustices that we, as neuroscientists and genome scientists, must be diligent not to repeat. Efforts to thoughtfully and respectfully engage with Indigenous communities have resulted in the development of several guidelines and strategies. In 2007, Indigenous Peoples' right to self-determination was internationally recognized in the United Nations Declaration on the Rights of Indigenous Peoples (UNDRIP) (45), which includes the right of Indigenous peoples to maintain, control, and protect their genetic resources (Article 31, p.11). In 2019, the Collective Benefit, Authority to Control, Responsibility, Ethics (CARE) Principles for Indigenous Data Governance were developed to advance Indigenous Peoples' governance of their own data in response to the growing desire for open science and data sharing (https://www.gida-global.org/care). Some Indigenous communities, such as the aforementioned San people of South Africa, developed their own policies to promote responsible research conduct and ensure Indigenous data are protected. In Canada, the OCAP Principles of ownership, control, access, and possession were developed to support data sovereignty for First Nations, providing guidelines on how First Nations data should be collected, protected, used, and shared (https://fnigc.ca). Similarly, in the United States, guidelines generated specifically for genomics research within Indigenous communities highlight important principles for ethical and respectful engagement (46, 47).

These existing guidelines provide high-level directives for Indigenous community research that should occur in tandem with institutional or community REBs. Yet wastewater sampling from Indigenous communities is a relatively new epidemiological approach that poses a unique set of ethical issues, underscoring the need for a comprehensive, robust, and ethical policy for WBE research that protects privacy, confidentiality, and data integrity while respecting Indigenous sovereignty. As WBE is also not currently evaluated by REBs, the potential risks for misuse of wastewater samples and any community data derived from those samples remain high, and we argue that all WBE studies involving Indigenous communities should continue only under REB oversight.

As Indigenous communities are diverse, the policy or framework should outline overarching principles and guidelines rather than prescriptive actions, but with full consideration of the specific issues surrounding WBE. Before their development, researchers should fully understand existing national and international governance frameworks for genomic data stewardship, particularly focusing on the unique challenges related to wastewater sources and Indigenous communities. Throughout, the policy should emphasize core principles of self-determination (Figure 1), that is, choice, partnership, and governance, and should include the following elements:

Respect Transparency and Cultural Humility

Listening and respect when working with Indigenous communities is paramount, and researchers should be mindful of when to step back so Indigenous ways of knowing and doing take precedence. There is a collective responsibility to ensure that the outcomes of genomics studies derived from Indigenous peoples prioritize the benefits to those communities, and there should be transparency in all things, including sample use, research findings and dissemination, and commercialization potential.

Notably, there should be both cultural competency and humility. Researchers should first demonstrate competence by doing their research on the communities they wish to engage with. They should appreciate that there will likely be differing ideas and points of view. The cultural significance of personal and biological (genetic) information and each community's traditional knowledge and world views should always be respected. Throughout the collection and research process, researchers should also be sensitive and respectful of Indigenous ways of doing things to ensure sample collection, data analysis, and dissemination are conducted according to the specifications detailed by the community. To effectively decolonize community-engaged research, it is necessary that researchers also express cultural humility (48), which is an openness to learning that involves acknowledging others' values, beliefs, and experiences, listening without judgment, and is a process that seeks to redress power imbalances. It is considered a lifelong commitment that begins with honest self-reflection to understand one's own values and biases. Strategies for the incorporation of cultural humility into community-based research have been developed. Itchuaqiyaq *et al*. (49), for example, base their guidelines on the experience of a collaboration between Aqqaluk Trust, a tribal organization serving the Iñupiat of northwest Alaska, and interdisciplinary researchers at Virginia Tech in the United States. These high-level strategies include respecting community leadership, knowing yourself and adjusting to community needs, accepting your role, avoiding manipulation of the project, and maintaining connections and trust. The Iñupiat Elders Council also provided specific instruction surrounding humility (49). Researchers who show cultural humility do not infer that their own knowledge is superior to the communities they are working with, and they value Indigenous ways of knowing and doing equally alongside Euro-Western ways.

Community Engagement

Although WBE is not presently under REB oversight, researchers should still have an understanding of the community governing and ethics structures, including leadership and relevant boards or committees, before engagement. Once engaged, researchers should design the study, acquire the data, and disseminate the findings in full partnership with Indigenous communities, giving the time required to build trust through relationship building. This can be facilitated through established community engagement strategies, examples that include Community-Based Participatory Research (CBPR) (46), Two-Eyed Seeing (50, 51), or Kaupapa Māori Methodology (52). Though CBPR is not Indigenous-specific, all three approaches value the importance of community engagement, transparency, and involving members of a study population as active and equal participants in all phases of the research project. However, in more recent years, there has also been a push for Indigenous leadership to be included within

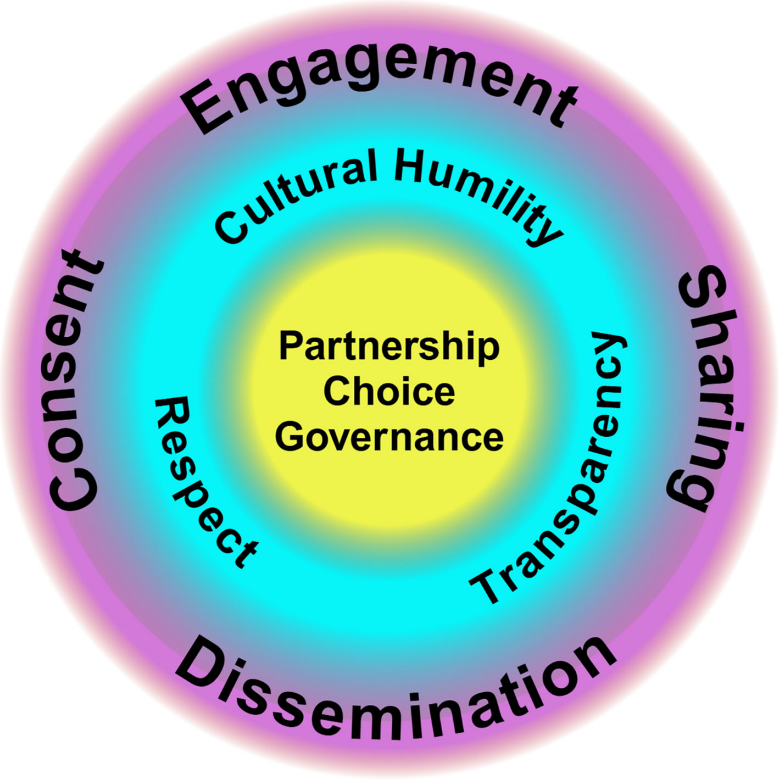

Figure 1. Guidelines for developing policy for ethical WBE research with Indigenous communities. The guidelines are built upon Indigenous self-determination, with partnership, choice, and governance as the foundational components. Researchers should exhibit respect, transparency, and cultural humility at all stages of the process. Guidelines should include recommendations for appropriate community engagement, sample and data sharing, consent, and knowledge dissemination. The image was generated with Biorender.

the research teams (e.g., academic, Elder, or Knowledge Keeper), which we strongly endorse.

Consent

Within the guidelines, a strategy for developing a robust consent process for wastewater sampling should be present, and the principle of Free, Prior, and Informed Consent (FPIC) should be followed. References to FPIC are found throughout UNDRIP (45), which emphasizes the importance of ensuring that Indigenous peoples' have effective and meaningful participation in decisions affects them, their communities, and their territories. More specifically, FPIC describes that consent must be given freely, without pressure or coercion, that sufficient time is given for communities to review all relevant information, and that communities have access to all the necessary information to determine the risks and benefits. The consent process should therefore be thorough with assurance that Indigenous communities fully understand the implications of wastewater sampling on their lands and the benefits and potential risks associated with human genomic DNA and/or drug residues in the samples.

In community research, consent must be obtained first from community leaders (e.g., Elders, Knowledge Keepers) before individuals are approached. This is particularly relevant to wastewater collection as samples are taken from community repositories and may contain the DNA of hundreds of individuals that would be subsequently analyzed as aggregated data. This poses additional challenges to consider, such as sampling locations and the possibility of individual and/or family privacy breaches. Indeed, the chances of identifying one individual from wastewater samples are extremely low but certainly not zero, leading to the question of who gives consent. Can one person have the authority to refuse sampling on community land due to the possibility of their DNA being in the samples? This question requires discussion far beyond what we can offer here. While some may argue that individual consent may not be the most practical approach, ultimately, that would be a community decision. On the other hand, if Indigenous leaders are chosen to consent on behalf of the community, it is essential to consider the possibility of community leaders changing with time. Therefore, specific timelines where consent would remain valid should be established, as wastewater surveillance generally takes place over long periods.

Sample Storage and Sharing

Implementing best practices for wastewater sample storage and sharing is essential to maximize the benefits of WBE while safeguarding ethical considerations, especially for Indigenous communities. One practical approach is to establish data sovereignty frameworks that empower communities to maintain control over their samples. For instance, community-led agreements could specify how samples are collected, stored, and used, ensuring alignment with local cultural and ethical standards. Co-designing sampling protocols, using a Two-Eyed Seeing approach to integrate Indigenous and Euro-Western ways of doing (50), for example, can ensure that collection practices align with Indigenous stewardship principles, emphasizing sustainability, reciprocity, and minimizing environmental disruption. Clear protocols, such as secure, anonymized storage systems, can protect individual and community privacy by deidentifying data before analysis. However, this would involve critical discussion as the community may want the samples returned. Additionally, a tiered consent process could be adopted, where communities provide informed consent for specific uses of their wastewater samples, preventing misuse or unauthorized sharing.

The sharing of wastewater samples should be governed by agreements prioritizing transparency and accountability. For example, researchers can adopt collaborative sample-sharing platforms where Indigenous representatives actively participate in decision-making. These platforms can ensure that the wastewater samples are used only for agreed-upon purposes and that results are reported back to the community in accessible formats. Regular audits of sample usage and storage practices can further

build trust and demonstrate adherence to ethical guidelines. Moreover, integrating traditional ecological knowledge into WBE practices can foster mutual respect and enrich public health strategies. By codifying these best practices into formalized policies, WBE can continue to advance public health goals while ensuring that communities, especially Indigenous populations, are respected and protected.

Data Governance and Knowledge Dissemination

Recommendations for Indigenous data governance should also be prioritized, ensuring that Indigenous communities are central in decisions affecting their information. Protocols surrounding data sharing should also be present. This is particularly important as open science remains a significant deterrent for Indigenous peoples for several reasons, including unrestricted access to personal samples and data, an overall inability for Indigenous people to govern their own personal information (53), and the substantial commercialization potential arising from their data alongside a lack of benefit to the communities themselves. Given that the data are aggregated, ownership of the data to the community should be clear. Finally, relationships with the communities should be maintained once the research is completed. There should be ongoing consultation with the communities regarding dissemination of the findings, and consideration of best practices for knowledge sharing should be prioritized for each community. Communities should be informed of and have the opportunity to review all findings before disseminating them to the public, government, researchers, and any other external stakeholders.

Conclusions

Emerging technologies in wastewater analysis, such as advanced metagenomics, real-time biosensors, and WBE, have revolutionized the monitoring of public health, environmental pollutants, and microbial communities. However, these advancements raise critical concerns regarding Indigenous data sovereignty and privacy. The granular data obtained from WBE can inadvertently capture sensitive information about specific communities, including genetic material and health indicators, which may conflict with Indigenous data governance principles. The ethical and legal challenges posed by the datafication of wastewater emphasize the need for robust frameworks to protect community-level data (54). Similarly, ethical engagement with Indigenous communities when deploying WBE technologies advocating for culturally sensitive practices that respect Indigenous data sovereignty, is imperative (55). There needs to be a balance between open scientific data and the privacy concerns of marginalized groups, highlighting the acute need for transparent data management policies that align with Indigenous rights (56). REBs and oversight committees at all levels—local, academic, and governmental—must incorporate WBE ethical guidelines into their standard protocols. This integration is essential to safeguard Indigenous communities from exploitative research practices and ensure their fundamental right to control their personal data.

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Author Contributions

M.L.P. and L.D.G conceived the manuscript topic and wrote the paper. M.L.P. generated the figures. All authors have read and approved the manuscript. Both authors take full responsibility for all figures and text and approve the content and submission of the study. No related work is under consideration elsewhere.

Corresponding authors: M.L.P. for the Indigenous aspects of the work and L.D.G. for queries associated with WBE. These corresponding authors take full responsibility for the submission process.

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References

1. Gatt JM, Burton KL, Williams LM, Schofield PR. Specific and common genes implicated across major mental disorders: a review of meta-analysis studies. J Psychiatr Res. 2015;60:1–13. DOI: 10.1016/j.jpsychires.2014.09.014. PMID: 25287955
2. Grove J, Ripke S, Als TD, Mattheisen M, Walters RK, Won H, et al. Identification of common genetic risk variants for autism spectrum disorder. Nat Genet. 2019;51(3):431–44. DOI: 10.1038/s41588-019-0344-8. PMID: 30804558; PMCID: PMC6454898
3. Meng X, Navoly G, Giannakopoulou O, Levey DF, Koller D, Pathak GA, et al. Multi-ancestry genome-wide association study of major depression aids locus discovery, fine mapping, gene prioritization and causal inference. Nat Genet. 2024;56(2):222–33. DOI: 10.1038/s41588-023-01596-4. PMID: 38177345; PMCID: PMC10864182
4. Trubetskoy V, Pardinas AF, Qi T, Panagiotaropoulou G, Awasthi S, Bigdeli TB, et al. Mapping genomic loci implicates genes and synaptic biology in schizophrenia. Nature. 2022;604(7906):502–8. DOI: 10.1038/s41586-022-04434-5. PMID: 35396580; PMCID: PMC9392466
5. Hatoum AS, Colbert SMC, Johnson EC, Huggett SB, Deak JD, Pathak G, et al. Multivariate genome-wide association meta-analysis of over 1 million subjects identifies loci underlying multiple substance use disorders. Nat Ment Health. 2023;1(3):210–23. DOI: 10.1038/s44220-023-00034-y. PMID: 37250466; PMCID: PMC10217792
6. Kowal E, Frederic G. Race, genetic determinism and the media: an exploratory study of media coverage of genetics and Indigenous Australians. Life Sci Soc Policy. 2012;8(2). DOI: 10.1186/1746-5354-8-2-1
7. Comfort N. Genetic determinism rides again. Nature. 2018;561(7724):461–3. DOI: 10.1038/d41586-018-06784-5. PMID: 30254357
8. Ehlers CL, Gizer IR. Evidence for a genetic component for substance dependence in native americans. Am J Psychiatry. 2013;170(2):154–64. DOI: 10.1176/appi.ajp.2012.12010113. PMID: 23377636; PMCID: PMC3603686
9. Bivins A, North D, Ahmad A, Ahmed W, Alm E, Been F, et al. Wastewater-based epidemiology: global collaborative to maximize contributions in the fight against COVID-19. Environ Sci Technol. 2020;54(13):7754–7. DOI: 10.1021/acs.est.0c02388. PMID: 32530639
10. Olesen SW, Imakaev M, Duvallet C. Making waves: defining the lead time of wastewater-based epidemiology for COVID-19. Water Res. 2021;202:117433. DOI: 10.1016/j.watres.2021.117433. PMID: 34304074; PMCID: PMC8282235
11. Choi PM, Tscharke BJ, Donner E, O'Brien JW, Grant SC, Kaserzon SL, et al. Wastewater-based epidemiology biomarkers: past, present and future. TrAC Trends Anal Chem. 2018;105:453–69. DOI: 10.1016/j.trac.2018.06.004
12. Gonzalez R, Curtis K, Bivins A, Bibby K, Weir MH, Yetka K, et al. Covid-19 surveillance in southeastern Virginia using wastewater-based epidemiology. Water Res. 2020;186:116296. DOI: 10.1016/j.watres.2020.116296. PMID: 32841929; PMCID: PMC7424388
13. Rousis NI, Gracia-Lor E, Zuccato E, Bade R, Baz-Lomba JA, Castrignano E, et al. Wastewater-based epidemiology to assess pan-European pesticide exposure. Water Res. 2017;121:270–9. DOI: 10.1016/j.watres.2017.05.044. PMID: 28554112
14. Farkas K, Fletcher J, Oxley J, Ridding N, Williams RC, Woodhall N, et al. Implications of long-term sample storage on the recovery of viruses from wastewater and biobanking. Water Res. 2024;265:122209. DOI: 10.1016/j.watres.2024.122209. PMID: 39126986
15. Acharya K, Halla FF, Massawa SM, Mgana SM, Komar T, Davenport RJ, et al. Chlorination effects on DNA based characterization of water microbiomes and implications for the interpretation of data from disinfected systems. J Environ Manage. 2020;276:111319. DOI: 10.1016/j.jenvman.2020.111319. PMID: 32889498
16. Whitmore L, McCauley M, Farrell JA, Stammnitz MR, Koda SA, Mashkour N, et al. Inadvertent human genomic bycatch and intentional capture raise beneficial applications and ethical concerns with environmental DNA. Nat Ecol Evol. 2023;7(6):873–88. DOI: 10.1038/s41559-023-02056-2. PMID: 37188965; PMCID: PMC10250199
17. Lorenzo M, Picó Y. Wastewater-based epidemiology: current status and future prospects. Curr Opin Environmen Sci Health. 2019;9:77–84. DOI: 10.1016/j.coesh.2019.05.007
18. Singer AC, Thompson JR, Filho CRM, Street R, Li X, Castiglioni S, et al. A world of wastewater-based epidemiology. Nature Water. 2023;1(5):408–15. DOI: 10.1038/s44221-023-00083-8

19. Kirmayer LJ, Brass GM, Tait CL. The mental health of Aboriginal peoples: transformations of identity and community. Can J Psychiatry. 2000;45(7):607–16. DOI: 10.1177/070674370004500702. PMID: 11056823
20. Kong C, Singh I. The ethics of global psychiatric genomics: multilayered challenges to integrating genomics in global mental health and disability-a position paper of the Oxford global initiative in neuropsychiatric genethics (neurogene). Am J Med Genet B Neuropsychiatr Genet. 2019;180(8):533–42. DOI: 10.1002/ajmg.b.32697. PMID: 30523674
21. Hyett SL, Gabel C, Marjerrison S, Schwartz L. Deficit-based indigenous health research and the stereotyping of indigenous peoples. Canadian J Bioeth. 2019;2(2):102–9. DOI: 10.7202/1065690ar
22. Dodson M, Williamson R. Indigenous peoples and the morality of the human genome diversity project. J Med Ethics. 1999;25(2):204–8. DOI: 10.1136/jme.25.2.204. PMID: 10226929; PMCID: PMC479208
23. Cunningham H. Colonial encounters in postcolonial contexts: patenting indigenous DNA and the human genome diversity project. Critique Anthropol. 1998;18(2):205–33. DOI: 10.1177/0308275x9801800205
24. MacIntosh C. Indigenous self-determination and research on human genetic material: a consideration of the relevance of debates on patents and informed consent, and the political demands on researchers. Health Law J. 2006;13:213–51. PMID: 17044328
25. Perbal L. The 'warrior gene' and the Maori people: the responsibility of the geneticists. Bioethics. 2013;27(7):382–7. DOI: 10.1111/j.1467-8519.2012.01970.x. PMID: 22494506
26. Dalton R. When two tribes go to war. Nature. 2004;430(6999):500–2. DOI: 10.1038/430500a. PMID: 15282577
27. McInnes RR. 2010 presidential address: culture: the silent language geneticists must learn–genetic research with indigenous populations. Am J Hum Genet. 2011;88(3):254–61. DOI: 10.1016/j.ajhg.2011.02.014. PMID: 21516613; PMCID: PMC3059421
28. Sterling RL. Genetic research among the Havasupai—a cautionary tale. Virtual Mentor. 2011;13(2):113–7. DOI: 10.1001/virtualmentor.2011.13.2.hlaw1-1102. PMID: 23121851
29. Garrison NA. Genomic justice for native Americans: impact of the Havasupai case on genetic research. Sci Technol Human Values. 2013;38(2):201–23. DOI: 10.1177/0162243912470009. PMID: 28216801; PMCID: PMC5310710
30. Pacheco CM, Daley SM, Brown T, Filippi M, Greiner KA, Daley CM. Moving forward: breaking the cycle of mistrust between American Indians and researchers. Am J Public Health. 2013;103(12):2152–9. DOI: 10.2105/AJPH.2013.301480. PMID: 24134368; PMCID: PMC3828980
31. Cann HM, de Toma C, Cazes L, Legrand MF, Morel V, Piouffre L, et al. A human genome diversity cell line panel. Science. 2002;296(5566):261–2. DOI: 10.1126/science.296.5566.261b. PMID: 11954565
32. Cavalli-Sforza LL, Wilson AC, Cantor CR, Cook-Deegan RM, King MC. Call for a worldwide survey of human genetic diversity: a vanishing opportunity for the human genome project. Genomics. 1991;11(2):490–1. DOI: 10.1016/0888-7543(91)90169-f. PMID: 1769670
33. Greely HT. Human genome diversity: what about the other human genome project? Nat Rev Genet. 2001;2(3):222–7. DOI: 10.1038/35056071. PMID: 11256074
34. TallBear K. Narratives of race and indigeneity in the genographic project. J Law Med Ethics. 2007;35(3):412–24. DOI: 10.1111/j.1748-720X.2007.00164.x. PMID: 17714251
35. Pullman D, Arbour L. Genetic research and culture: where does the offense lie? In: Young JO, Brunk CG, editors. The Ethics of Cultural Appropriation. West Sussex, UK: Wiley-Blackwell; 2009. p. 115–39.
36. Hook GR. "Warrior genes" and the disease of being māori. Mai Review. 2009;2:1–11. Available online at: https://www.journal.mai.ac.nz/system/files/maireview/222-1507-1-PB.pdf
37. Merriman T, Cameron V. Risk-taking: behind the warrior gene story. N Z Med J. 2007;120(1250):U2240. PMID: 17339896
38. Raine A. From genes to brain to antisocial behavior. Curr Dir Psychol Sci. 2008;17(5):323–8. DOI: 10.1111/j.1467-8721.2008.00599.x
39. Gillett G, Tamatea AJ. The warrior gene: epigenetic considerations. New Genet Soc. 2011;31(1):41–53. DOI: 10.1080/14636778.2011.597982
40. Schuster SC, Miller W, Ratan A, Tomsho LP, Giardine B, Kasson LR, et al. Complete Khoisan and Bantu genomes from southern Africa. Nature. 2010;463(7283):943–7. DOI: 10.1038/nature08795. PMID: 20164927; PMCID: PMC3890430
41. Chennells R, Steenkamp A. International genomics research involving the San people. In: Schroeder D, Cook J, Hirsch F, Fenet S, Muthuswamy V, editors. Ethics Dumping. Springerbriefs in Research and Innovation Governance. Cham: Springer; 2018. p. 15–22.
42. Schroeder D, Chatfield K, Singh M, Chennells R, Herissone-Kelly P. The San code of research ethics. In: Equitable Research Partnerships. SpringerBriefs in Research and Innovation Governance. Cham: Springer; 2019. p. 73–87.
43. Jacobs D, McDaniel T, Varsani A, Halden RU, Forrest S, Lee H. Wastewater monitoring raises privacy and ethical considerations. IEEE Transact Technol Soc. 2021;2(3):116–21. DOI: 10.1109/tts.2021.3073886
44. Tindana P, Molyneux CS, Bull S, Parker M. Ethical issues in the export, storage and reuse of human biological samples in biomedical research: perspectives of key stakeholders in Ghana and Kenya. BMC Med Ethics. 2014;15:76. DOI: 10.1186/1472-6939-15-76. PMID: 25326753; PMCID: PMC4210627
45. United Nations General Assembly. United Nations Declaration on the Rights of Indigenous Peoples: Resolution/Adopted by the General Assembly. 2007. Available online at: https://www.un.org/development/desa/indigenouspeoples/wp-content/uploads/sites/19/2018/11/UNDRIP_E_web.pdf (accessed on Dec 12, 2024).
46. Claw KG, Anderson MZ, Begay RL, Tsosie KS, Fox K, Garrison NA, et al. A framework for enhancing ethical genomic research with indigenous communities. Nat Commun. 2018;9(1):2957. DOI: 10.1038/s41467-018-05188-3. PMID: 30054469; PMCID: PMC6063854
47. Barton KS, Porter KM, Mai T, Claw KG, Hiratsuka VY, Carroll SR, et al. Genetic research within indigenous communities: engagement opportunities and pathways forward. Genet Med. 2024;26(7):101158. DOI: 10.1016/j.gim.2024.101158. PMID: 38699966
48. Hurley DA, Kostelecky SR, Townsend L. Cultural Humility. Chicago, IL: ALA Editions; 2022.
49. Itchuaqiyaq CU, Lindgren CA, Kramer CQ. Decolonizing community-engaged research: designing CER with cultural humility as a foundational value. Commun Des Quart. 2023;11(3):12–20. DOI: 10.1145/3592367.3592369
50. Illes J, Perreault ML, Bassil K, Taylor-Bragge R, Bjaalie J, Chneiweiss H, et al. Two-eyed seeing and other indigenous perspectives for neuroscience. Nature. 2025;638(8049):58–68. DOI: 10.1038/s41586-024-08437-2. PMID: 39910384
51. Wright AL, Gabel C, Ballantyne M, Jack SM, Wahoush O. Using two-eyed seeing in research with indigenous people: an integrative review. Int J Qual Methods. 2019;18. DOI: 10.1177/1609406919869695
52. Hiha AA. Kaupapa māori methodology: trusting the methodology through thick and thin. Aust J Indig Educ. 2015;45(2):129–38. DOI: 10.1017/jie.2015.30
53. Fox K. The illusion of inclusion – the "all of us" research program and indigenous peoples' DNA. N Engl J Med. 2020;383(5):411–3. DOI: 10.1056/NEJMp1915987. PMID: 32726527
54. Scassa T, Robinson P, Mosoff R. The datafication of wastewater: legal, ethical and civic considerations. Technol Regulat. 2022;2022:23–35. DOI: 10.26116/techreg.2022.003
55. Manning S, Walton M. Ethical and responsible development of wastewater-based epidemiology technologies. Institute of Environmental Science and Research; 2021.
56. Zipper SC, Whitney KS, Deines JM, Befus KM, Bhatia U, Albers SJ, et al. Balancing open science and data privacy in the water sciences. Water Resour Res. 2019;55(7):5202–11. DOI: 10.1029/2019wr025080

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THOUGHT LEADERS INVITED REVIEW

Translating stress systems: corticotropin releasing factor, its receptors, and the dopamine system in nonhuman primate models

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Stress is a fact of life, affecting organisms from the smallest invertebrates to humans. Mediating the stress system is the ancient neuropeptide, corticotropin releasing factor (CRF), which works as a neuromodulator to alter brain systems and homeostatic responses to stress. In humans, many stress-related psychiatric illnesses are linked to dysregulation of monoamine systems, which have cross-talk with CRF-enriched circuits. In this review, we focus on the CRF and the midbrain dopamine (DA) system, particularly as it relates to the nonhuman primate. While tremendous knowledge of CRF-DA mechanisms has been gleaned from rodent models, treatments for stress-related diseases have been elusive, raising the question of whether higher animal models might be required. Subtle shifts in CRF peptide or CRF receptor localization, and the expansion and complexity of DA neuron populations, may hold some of the keys to understanding long-standing stress effects on the DA system in humans. Our laboratory has especially been interested in laying out the neural architecture of the CRF-DA system interface in the nonhuman primate, as a close anatomic model for human. Using rodent models as a starting point, we describe aspects of this complex system that inform our understanding of CRF-DA interactions, and focus on results that have been, and those that still need to be, translated to nonhuman primate models.

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Anatomy of the CRF Stress System and DA Neurons

As a key driver of the hypothalamic pituitary axis (HPA) in modulating behavioral stress responses, corticotropin-releasing factor (CRF) [also known as corticotropin-releasing hormone (CRH)] is a 41-amino acid peptide that was first discovered in the rat hypothalamus over 40 years ago (1). This peptide, like all neuropeptides, is packaged with classic "fast" neurotransmitters and acts as a neuromodulator. Working in concert with its primary transmitters, CRF has unique modes of action that can shape stress circuitry throughout the brain. Although there is a vast literature in rodent models, CRF mRNA and its peptide were subsequently identified throughout the brain in human and nonhuman primates, as well as in rodent models, Review (2). In human, there is also considerable interest in CRF receptors that are found in other organ systems, including the immune (3), reproductive (4), metabolic (5), and cardiovascular systems (6, 7) due to known stress effects on these systems and stress-related disease. Because stress, broadly defined, is implicated in the onset and recurrence of numerous neuropsychiatric illnesses (8–11), CRF brain targets continue to be a subject of inquiry.

Cross-species studies indicate that CRF mRNA and protein cellular distribution in the brain shares similarities but also important difference between rodents and nonhuman primates (2). One purpose of this review is to highlight some of the known brain differences between the species. Areas of dense distribution of CRF neurons in both nonhuman primates and rodents include the "central extended amygdala" and ventral pallidum (12–15). Species differences include a generally more diffuse distribution of CRF-containing cells in primate hypothalamus and extended amygdala, and more discrete clusters within these regions in rodents, see Review for details (Figure 1) (2, 14, 16). CRF-containing neurons are found in many areas outside the extended amygdala, which have also been implicated in anxiety-like behavior and/or sensory responses (particularly in the auditory system). These regions include the periaqueductal gray, peripeduncular nucleus, pedunculopontine tegmental nucleus, parabrachial nucleus, locus coeruleus (LC), lateral dorsal tegmental nucleus (LDTg), median raphe nucleus, and pontine reticular nuclei.

This review will also focus on the way CRF neurons influence a broadened and more elaborate dopamine (DA) system in the primate. Stress effects leading to excess DA release are well-documented across species, but there is little known about the cellular and circuit basis of this, especially in higher primates. The "central extended amygdala," a structural continuity of the central amygdala nucleus through cell columns in the forebrain with the lateral bed nucleus of the stria terminalis, that is enriched in CRF-containing neurons, has been one focus (17, 18). Central "extended amygdala" CRF pathways are well known to project to the DA system (19–21), and considered a means by which stress alters DA release, and affects motivated and higher cognitive behaviors (22–27). Outside of the well-studied extended amygdala, however, there are few studies documenting other CRF-modulated paths to the DA system [although see Soden (28) and Chang (29)], which can be assumed to have specific targets and affect specific systems. The mechanisms of how CRF release in general influences DA firing also remains elusive (30, 31). As noted, knowledge of the array of cell type–specific CRF inputs, and the complexity of postsynaptic cellular targets found in the ventral midbrain are missing pieces of the puzzle. This is particularly true when it comes to understanding higher species.

One clear piece of evidence is that there are high concentrations of CRF-positive axons in the midbrain DA system in both rodents and primates (12, 13, 32–34). In both species, CRF-containing fibers overlap the midline ventral tegmental area (VTA), and extend into the lateral VTA (pigmented parabrachial nucleus, PBP) and into the retrorubral field (RRF, or A8 group). These latter, non-midline, regions are especially well-developed in primates (35, 36) and receive an abundance of CRF terminals (Figure 2).

CRF is Packaged as a Neuromodulator

A key level of complexity for understanding CRF actions is that CRF is considered a "neuromodulator" instead of a "neurotransmitter." Like most peptides, it does not directly elicit an action potential (37). Instead, CRF amplifies or attenuates the excitatory/inhibitory function of transmitters

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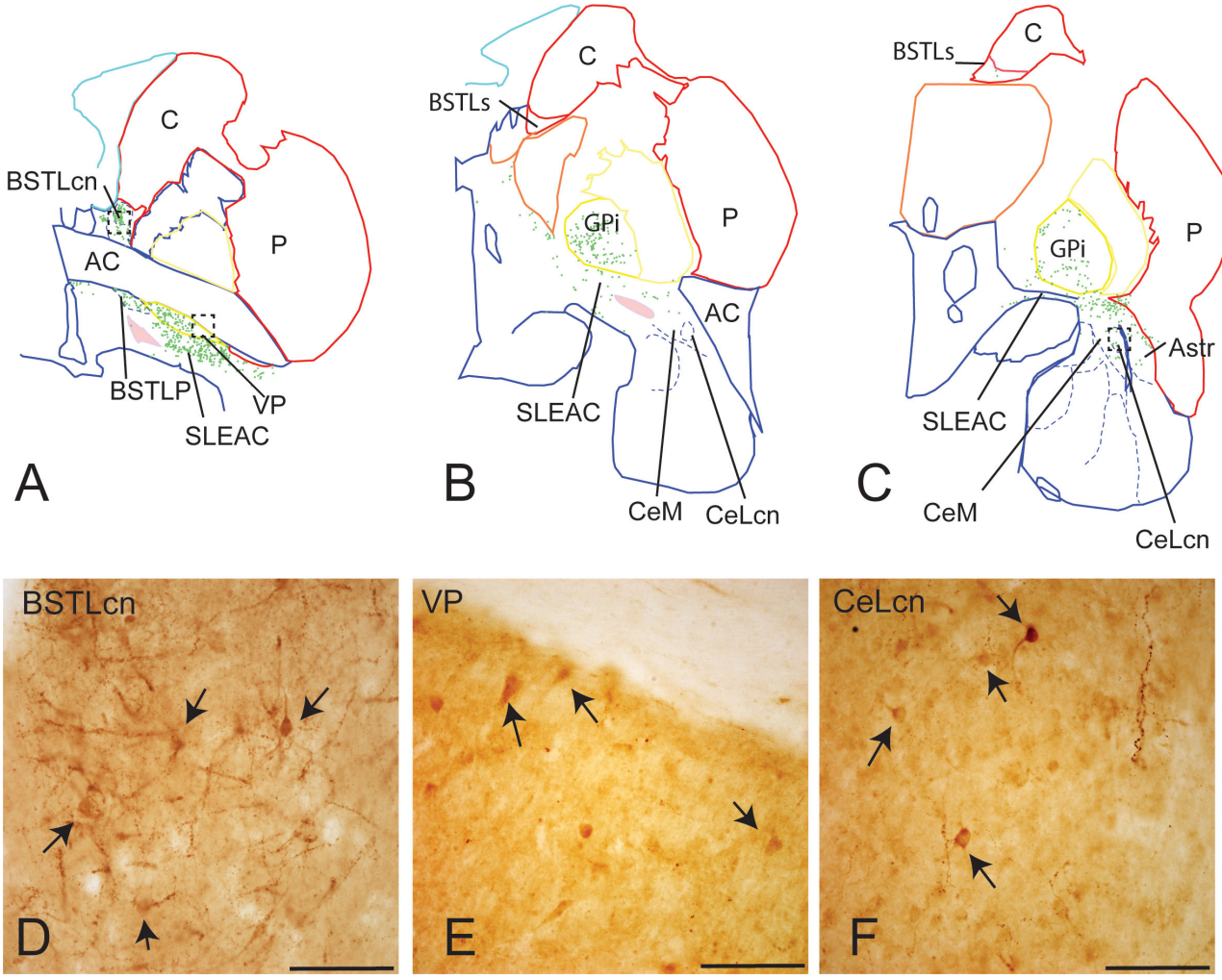

Figure 1. (A–C) Distribution of CRF-immunoreactive neurons in the nonhuman primate CEA and VP subregions. Labeled neurons are distributed broadly and extend from the ventral striatum through the caudal central nucleus. Colored lines indicate various surrounding structures; red: striatum, cyan: ventricles, yellow: globus pallidus, orange: thalamus, pink: cholinergic cell clusters, green: CRF-positive cells. (D) High-powered micrographs of CRF-labeled cells and processes in BSTLcn, corresponding to boxed area in A. (E) High-powered micrographs of CRF-labeled cells and processes in VP, corresponding to boxed area in A. (F) High-powered micrographs of CRF-labeled cells and processes in CeLcn, corresponding to boxed area in C. Scale bar = 100 μm. Abbreviations: AC, anterior commissure; Astr, amygdalostriatal area; BSTLcn, bed nucleus of the stria terminalis; lateral central subdivision; BSTLP, bed nucleus of the stria terminalis, ventral posterior subdivision; C, caudate nucleus, CeLcn, central nucleus, lateral central subdivision; CEM, central nucleus, medial subdivision; P, putamen; SLEAc, sublenticular extended amygdala, central subdivision; VP, ventral pallidum. *Figure used and modified with permission from (14).*

released with it (38, 39). Therefore, CRF's specific actions at terminals must be considered in the context of the classic fast transmitters including glutamate and/or GABA, with which it is packaged in specific circuits (14, 37, 40). In both primates and rodents, CRF + axon terminals exhibit synaptic profiles in the ventral midbrain based on electron microscopic (EM) studies (33, 34). These terminals are filled with both CRF + dense core vesicles and a more diffuse immunostaining that surrounds clear (fast transmitter) vesicles. The physiologic impact of increased CRF release in response to stress is thought to regulate the excitatory/inhibitory output of its circuit in a duration-dependent manner (41). Complicating the picture, however, is the fact that while CRF is released at classical synapses and binds presynaptic and postsynaptic membrane receptors, it can also be released through exocytosis (also known as "volumetric" or "extrasynaptic" release) directly through the axon membrane into the extracellular space (42). Therefore, while documenting the distribution and characterization of CRF + axon terminals is important for understanding co-modulation of classic transmitters in afferent sources, the location of CRF receptors is also needed to help to clarify which cell types are affected by both synaptic and "extrasynaptic" CRF release.

CRFR1 and CRFR2

The biological actions of CRF at postsynaptic targets are mediated through at least two known receptors that have been identified in rat, primate, and human brains: CRF receptor type 1 (CRFR1) and CRF receptor type 2 (CRFR2) (43). Both receptor subtypes are members of the G protein–coupled receptor family and although they share significant sequence homology, they are pharmacologically and anatomically distinct (44–46). Both the CRFR1 and CRFR2 receptors are coupled with stimulatory G proteins, which undergo specific conformational changes depending on ligand binding (47). CRF bind CRFR1 and CRFR2 with differing affinities, with CRF having a high affinity for CRFR1 and only a moderate affinity for CRFR2. Urocortins, identified after the discovery of CRF, share structural similarities with CRF (48–50), and either bind exclusively to CRFR2 (UCN II, UNC III), or have a higher affinity for CRFR2 (UCN I) (49).

Early electrophysiological and pharmacological studies in rodents demonstrated fundamental differences between CRFR1 and CRFR2 receptors, which have been reviewed extensively (43, 51–53). Generalized CRFR1 antagonism (via intracerebroventricular injection of the CRFR1 antagonist, antalarmin) (54) and CRFR1 knockout (55, 56) suggested

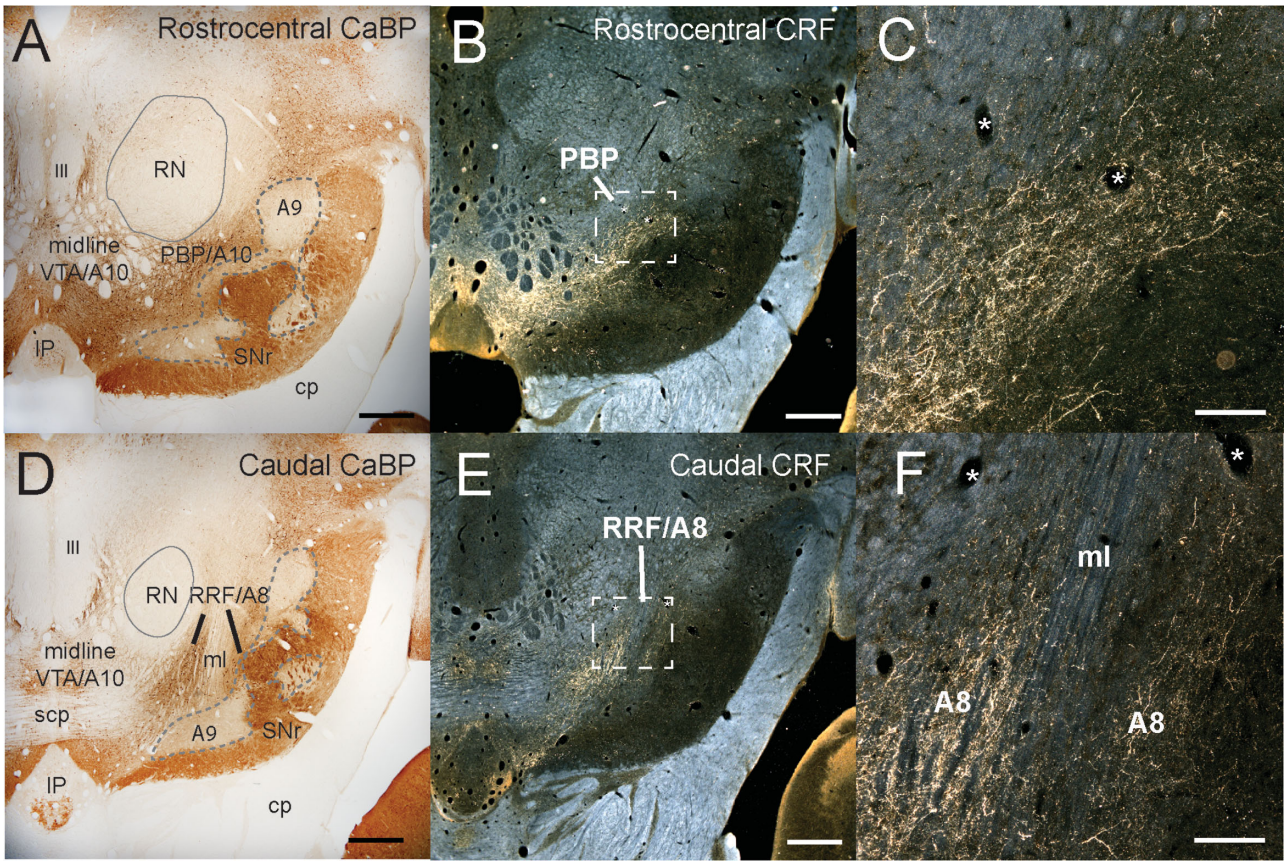

Figure 2. Localization of CRF-immunoreactivity in ventral midbrain. (A) low magnification brightfield micrograph of the ventral midbrain rostrocentral level visualized with CABP-IR, a marker of the A10 and A8 neurons. CABP-positive A10 neurons contrast with CABP-negative SNc/A9 neurons, outlined with dotted lines. (B) CRF-IR in fibers in adjacent sections, visualized with darkfield microscopy. (C) A higher magnification of boxed region in B showing patches of thin beaded CRF-positive fibers in a section of the PBP. (D) Low-magnification brightfield micrograph of CaBP-IR in the caudal ventral midbrain. CaBP-IR neurons are found in the A10 and RRF/A8 and absent in SNc/A9 subregion (dotted line). (E) CRF-IR in neighboring section to D, seen under darkfield magnification. (F) CRF-labeled fibers course through the RRF/A8, which is bisected by the medial lemniscus (ml) seen under higher magnification, boxed region in E. Scale bar = 1 mm (A, B, D, E), 250 μm (C, F). *Abbreviations:* III, third nerve; CRF, corticotropin-releasing factor; CaBP, calbindin; IP, interpeduncular nucleus; cp, cerebral peduncle; scp, superior cerebellar peduncle; ml, medial lemniscus; SNr, substantia nigra reticulata; PBP, parabrachial pigmented nucleus; RN, red nucleus; VTA, ventral tegmental area. *Figure used with permission from (33).*

anxiogenic effects. Early studies also suggested a general opposing role for CRFR1 and CRFR2, but it is now generally acknowledged that this is an overly simplistic view (57). Subsequent studies using more advanced techniques such as conditional and site-specific receptor manipulations indicate a dependence on circuit and cell type–specific characteristics for CRF receptor action. For example, it is now known that low levels of anxiety after enriched housing are associated with low levels of CRFR1 mRNA in the amygdala, and amygdala-specific CRFR1 knockdown reduces anxiety (58). In contrast, CRFR1 knockdown in the globus pallidus (GP) increases anxiety-like behavior, suggesting an anxiolytic role in GP circuitry (59).

CRFR1 and CRFR2 mRNA and Protein Distribution: Some Species Differences

Some of the earliest CRF-binding assays were performed in monkey, and revealed intense CRF-binding occurring widely throughout the cortex, amygdala, hippocampus, and cerebellum, in addition to selected thalamic, hypothalamic and brainstem nuclei (60, 61). However, to date, very little is known of the actual distribution of the CRF receptors in the nonhuman primate. Sanchez *et al.* (1999) mapped the neuroanatomic distribution of CRFR1 and CRFR2 receptor subtypes in the adult rhesus monkey utilizing *in situ* hybridization and also receptor autoradiography (62). Noting some differences between the two techniques, CRFR1 mRNA was abundant throughout the cortex (including prefrontal, cingulate, insular, parietal, and temporal neocortical areas), dentate gyrus of the hippocampus, several amygdaloid subnuclei, cerebellar granule cell layer, pituitary, and LC. High densities of CRFR2 were found in neocortical areas (prefrontal, striate, cingulate, and insular cortices), CA1 of the hippocampal formation, the choroid plexus, the paraventricular hypothalamic nucleus, supraoptic nucleus, amygdala, pituitary, and mammillary bodies. Notably, many regions including the cortex, amygdala and hippocampus have strong distributions of both receptors in the primate. However, some brain regions had selective distributions of CRFR1 or CRFR2 mRNA, supporting the hypothesis that each receptor subtype may have distinct but complementary functional roles within the primate central nervous system (CNS). Unfortunately, the midbrain was not included in these results, leaving unaddressed the specific distribution or existence of these receptors in this critical region.

CRFR1 protein studies in nonhuman primate largely converged with the mRNA findings (63). CRFR1 peptide (AA 21-34) was generally localized in cell bodies and dendrites highlighting a strong postsynaptic role. In this broad survey of the monkey brain, CRFR1 immunoreactivity was also present in regions of the cerebral cortex, basal forebrain, basal ganglia, thalamus, and cerebellum converging with prior mRNA results (62). Importantly, CRFR1 was highly expressed in the ventral midbrain, particularly in the lateral substantia nigra and reticulata. No studies have conducted a similarly broad survey of CRFR2 expression in the nonhuman primate brain.

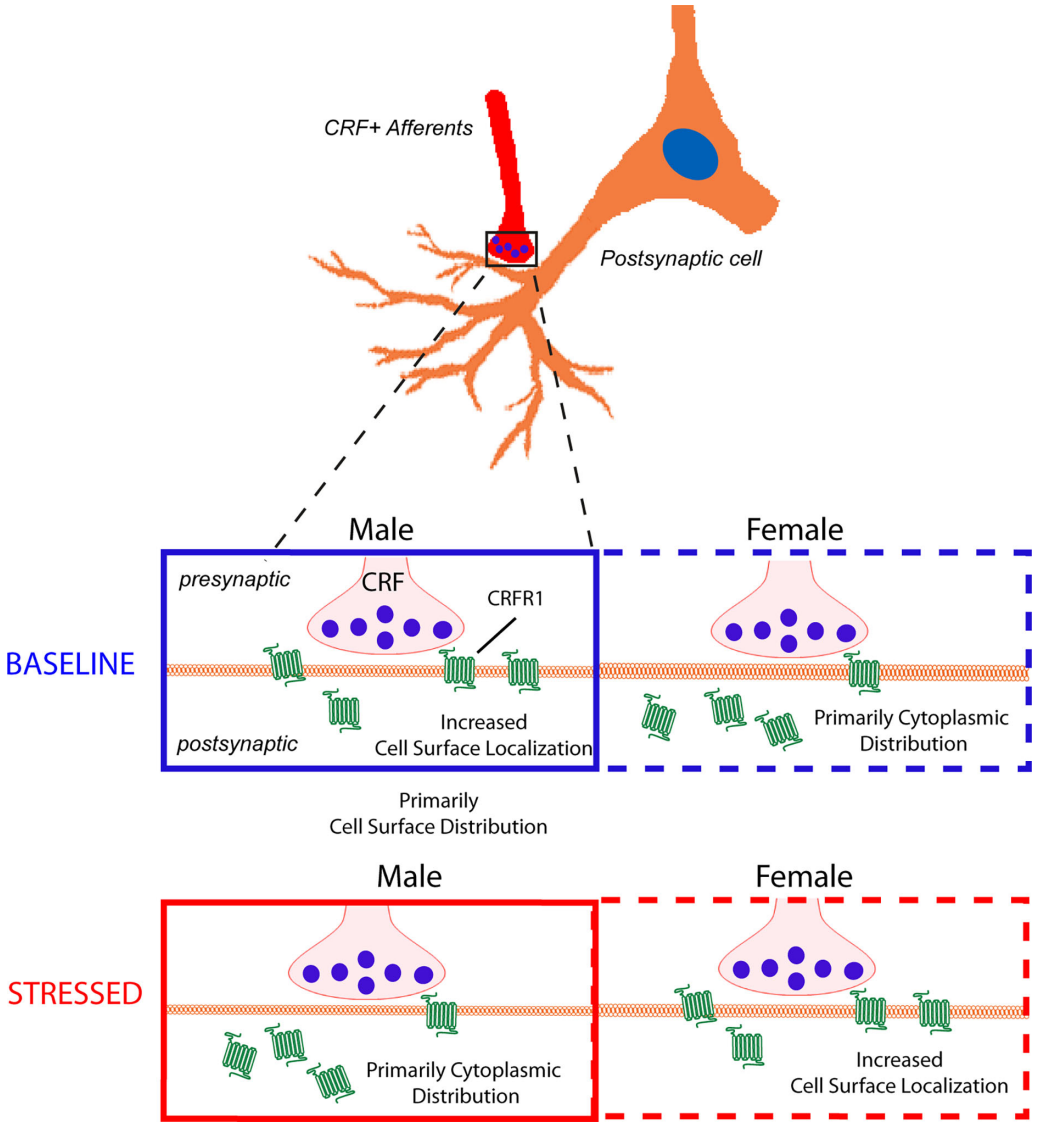

Figure 3. Differential regulation of CRF signaling during baseline and stressed situations in males versus females. At baseline, CRFR is primarily distributed at the cell surface in males versus a primarily cytoplasmic distribution in females. Following stress, CRFR is internalized in males versus relocation to the cell surface in females. *Figure used and modified with permission from* (68).

The general CRF receptor distribution in primates reveals some important differences compared to the rodent. While the human and nonhuman cortex express both CRFR1 and CRFR2 mRNA as noted above, the rodent cortex exclusively expresses CRFR1 mRNA (45, 64). Similarly, in human and monkey, both receptor subtypes are abundant in the pituitary (7, 62), and CRFR1 antagonists alone have relatively minor effects on ACTH or cortisol release in humans (65, 66). In rodent, CRFR1 receptors only are found on adrenotrophs in the anterior pituitary. Other discontinuities in the distributions of the two receptors exist between the species, suggesting possible differences in the balance of CRF receptor subtypes across brain regions, highlighting the importance of nonhuman primate models to improve our understanding of the human CRF system.

Presynaptic and Postsynaptic CRFR1 and CRFR2

Important EM work in rodents reveals that CRFR1R and CRFR2 exist presynaptically and postsynaptically, depending on the brain region assayed (67–71). Thus, CRF effects likely result from a balance of influence on presynaptic versus postsynaptic neural control via each receptor, which vary in a site- and sex-specific manner. CRFR1 is differentially expressed in some brain regions in females and males. Importantly, however, in brain regions without apparent sex differences in CRF receptor expression, differential receptor signaling/sensitivity may be at play (72, 73) Exogenous factors such as stress and pharmacologic manipulations can dynamically regulate CRF signaling with differing behavioral effects in each sex, possibly through differences in CRF receptor sensitivity and engagement with intracellular pathways. Depending on brain region and sex, significant trafficking of postsynaptic CRFR1 and CRFR2 receptors between the membrane and cytosol regulates the availability of the receptor for CRF postsynaptic effects (74, 75) (Figure 3). In brain regions with baseline sex differences in CRFR1 and CRFR2 receptor expression (68, 74), these differences can be exacerbated following stress via intracellular mechanisms that internalize and externalize (recruit) receptors (68). Thus, against a back-drop of sex differences, stress can be homeostatically regulated in each sex by receptor tracking, at least in selected brain regions (74). Knowledge of the basic anatomic locations of CRF receptors, and shifts across development and stress effects in the two sexes, is evolving.

CRFR1 and CRFR2 Gene Variants in Human Disease

An important issue for translational approaches is splice variation in CRF receptors, which have tissue specific distributions and vary by species (76). Alternative splicing is a known mechanism for regulatory control of signaling that can affect cellular function. Different isoforms of the

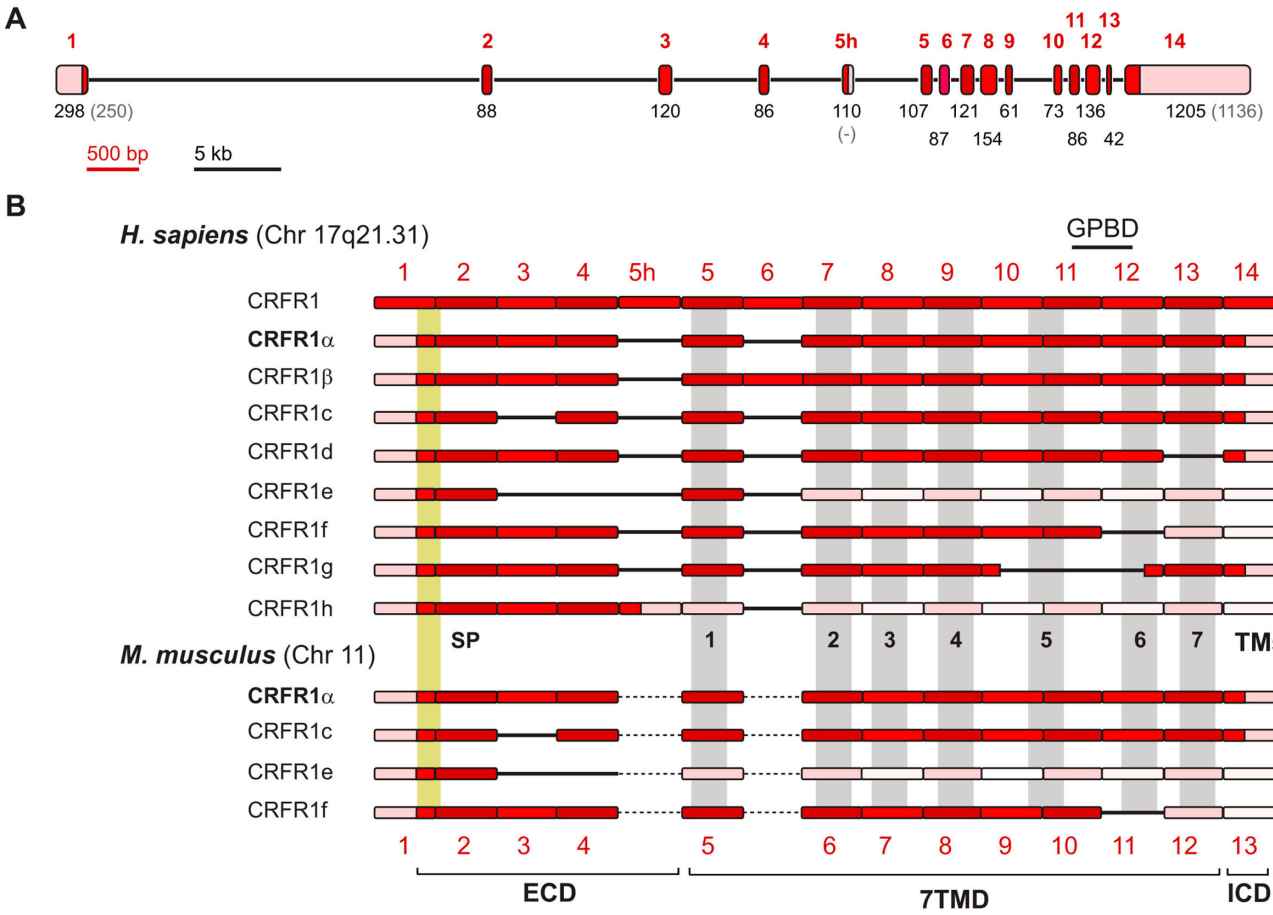

Figure 4. The CRFR1 gene and isoforms following alternative splicing. Note that the CRF1 gene is on different chromosomes in human and mouse. (A) Schematic of the human *CRFR1* gene structure, with base pair (bp) numbers of exons (red) indicated below them. Exons are the coding sequences present in the mature mRNA. Introns (noncoding sequences) are depicted with black lines. Deviations in the size of mouse exons (red) shown in parentheses and gray. (B) Human and murine *CRFR1* splice variants. Red color denotes exons, black color denotes introns, yellow denotes the exon segments encoding the signal peptide (SP). Exon segments coding for transmembrane helices (TMs 1-7) are denoted by gray bars. In both A. and B. pink denotes 5′ or 3′ untranslated regions. ECD = extracellular domain; GPBD = G protein–binding domain; ICD = intracellular domain. *Figure from (76) used with permission.*

receptor can be present in different tissues or cells. In the CRFR1 family, the CRFR1α variant is dominant but its activity is dependent on expression of other isoforms that may compete with or dimerize/oligomerize with the CRFR1α isoform, altering its activity. Human CRFR1 and CRFR2 genes are expressed on chromosomes 17 and 7, respectively, which contrasts with their location on chromosomes 11 and 6 in mouse. Both genes have multiple isoforms, as a result of alternative splicing particularly in human (76) (Figure 4). Gene variants of CRFR1 and CRFR2 are common and, in humans, specific variants are associated with different conditions that are regulated by stress: major depression, type II diabetes, polycystic ovary disease, and irritable bowel syndrome (5, 77–79). The role of CRF signaling in these disorders owes to effects on the HPA, and also to direct CRF effects on external tissues. The tremendous variety in CRFR1 human gene variants, and how they interact with environmental factors such as adverse life experience, has been a focus of psychiatric genetics for several decades (78). Early linkage studies looking for a role for CRF variants in mood and anxiety disorders were not compelling, however, mainly because they were underpowered (80). Nonetheless, the sum of genotyping clinical studies supports a role for CRFR1 in the pathophysiology of depression, anxiety disorders, and alcohol abuse, the latter of which is highly comorbid with anxiety and depression diagnoses. While it is understood that single-gene effects are unlikely in complex disorders, CRFR1 variants likely play a role in conjunction with other genetic risk factors, and also with environmental perturbations (81).

CRF Signaling and Behavior Through the Midbrain DA System

Stressful stimuli lead to dramatic adaptive changes in ongoing behaviors in order to shift the animal into more adaptive responses (82). Stressful manipulations such as footshocks, pinches, or airpuffs, and prolonged anxiogenic events (e.g., restraint), increase DA cell firing (83, 84), and result in DA release in striatum, amygdala, and prefrontal cortex (23–27, 85–87). Similarly, CRF stimulation in the midbrain generally increases DA neuron activity (88) and release at the terminal (89, 90). Thus, stress experience shapes DA signaling and behavior, including motivated behaviors, response to novelty and decision making (91–94). However, the mechanisms behind these effects, or differential effects on specific brain circuits, are far from clear.

In slice preparations, CRF excites VTA DA neurons in a bimodal, dose-dependent manner (88, 95–97). In part, CRF increases excitability by effecting release of calcium from intracellular stores in DA neurons (98). However, CRF promotes excitation of VTA DA neurons via both presynaptic and postsynaptic mechanisms involving both CRFR1 and CRFR2 (88, 96–101). Complicating this picture, CRF release can itself also alter the expression of CRFR1 and CRFR2 expression (102). Stress was recently shown to regulate CRFR1 expression in DA neurons, with consequences for both DA firing and behavior (103).

In vivo, most work has investigated how CRF modulates motivational and decision-making processes, including addictive behaviors. For example, both restraint stress and infusions of CRF into the VTA diminish

preference for larger rewards with a greater effort cost. Notably, CRF antagonism blocks these effects in stressed animals (104). Similar studies show that stress and CRF administration in the VTA reduce motivation to work for food reward behaviors, but regulate DA firing in a pathway- and stimulus-specific manner (105). This circuit-based approach makes clear that CRF effects on behavior and DA release are critically dependent on gating of specific active afferent/efferent pathways.

Stress likely also influences non-DA neuronal cells in the midbrain DA system, including GABAergic interneurons (106–110). These neurons are proposed to exert tight inhibitory control on DA neurons (108, 111, 112), but the effects of CRF on these neurons have not been studied in detail. CRF increases firing of inhibitory GABA neurons in the VTA, although it is unclear if this a causative or compensatory effect related to CRF-induced DA activation (95, 97). CRFR1 mRNA and CRF-binding protein (associated with CRF receptors) is expressed in some VTA GABAergic neurons per studies in rodent (103, 113). Therefore, to better understand the ways in which CRF differentially impacts the molecularly defined DA neuron subtypes as well as non-DA neurons in the midbrain DA system, anatomic localization of these subpopulations and their relative expression of CRF receptors is a missing piece of the puzzle.

The Midbrain DA Target is Ruled by Anatomic Complexity

The midbrain DA neurons are no longer considered a homogeneous system. DA neurons are physiologically heterogeneous with respect to both intrinsic firing and coding properties. For example, DA neuron pacemaking and spiking depend on a variety of ion channels, which vary across the DA subregions, giving rise to heterogeneity in spontaneous and induced-spiking activity (114, 115). Many recent papers show that physiologic activity, as well as molecular/transmitter content and circuit connections, can be predicted from mediolateral and rostrocaudal anatomy of ventral midbrain in both rodents and primates (116–122). This basic anatomy (based on developmental trajectories) provides an important organizational principle for predicting cell types and connections between the species. Although the primate ventral midbrain system is larger and more elaborate, the mediolateral and rostrocaudal axes provide important anchors for comparison.

The nonhuman primate ventral midbrain is an important, albeit understudied, bridge to understand human disorders because of its similarity to the human (123–125). Because DA neurons serve the individual over the lifespan, the nonhuman primate is also a closer model for understanding specific populations that are particularly plastic or that are vulnerable over long time periods, that is, in aging or chronic environmental insult. Fortunately, identification of the basic DA subregions (A10, A9, and A8) across species can be done with specific histochemical markers, permitting regional comparisons across species (126–128) (Figure 5).

The concept of DA neurons generating "reward prediction errors" in learning was first discovered in nonhuman primate, and became a dominant model of DA function (129, 130). As this concept has been debated and expanded, location-dependent roles of different DA subregions have been raised, with DA neurons in different midbrain regions involved in other functions. In monkey, laterally displaced DA neurons appear to have a separate role, signaling the biologic relevance (salience) of both reward and nonreward predicting stimuli (salience coding) (131, 132). These DA neurons may play a different role in complex behaviors such as orienting, or preparing strategies to avoid potentially aversive cues (84, 131, 133–135). This general medial-lateral trend has been noted as well in rodent studies, with different functional properties distributed along this axis (27, 136).

DA Subregions are a Translational Anchor for Understanding Heterogeneity

Although the vast majority of work on DA heterogeneity is in mouse and rat, the conserved organization of the DA subregions serve as important landmarks to approach higher species (137) (Figure 5). The nonhuman primate system is expanded in both mediolateral and rostrocaudal directions (Figure 5A and B, rostral; C and D caudal). Across rodents and primates, the A10 subregion, referred to as the VTA, contains a number of subnuclei. In addition to multiple midline subnuclei (mVTA), the A10 includes the lateral-most VTA subnucleus, the PBP. The PBP is disproportionately enlarged in higher primates (35). While previously referred to as the "dorsal A9," it is now clear that the primate lateral VTA (PBP) stretches dorsolaterally over the A9, comprising a large expanse of the midbrain. The entire A10 is closely related to the A8 subregion, and is continuous with it in macaque and human (see below) (Figure 5C and D). Both regions, in both rodent and primates (including human), have DA neurons that express calbindin-D28K, a calcium-binding protein (CaBP, Figure 5A, D, and E), which is absent in the A9. The A8 (also known as the RRF), is enlarged volumetrically in nonhuman primates (36, 138), and is also an important component of the mesolimbic path. The A9 is conspicuously lacking in CaBP, but is enriched in other protein markers as noted below.

Major Transmitters in the DA Subregions

All DA subregions contain dopaminergic, GABAergic, and glutamatergic neurons, with variable relative densities of transmitter-specific neurons in each region. This has been shown across mice, rats, and primates (139–143). In addition, "multiplexed" neurons that coexpress at least two of the aforementioned neurotransmitters are well described in rodents (142, 144–147), and are beginning to be identified in higher species. (Although not reviewed here, some DA neurons also coexpress coregulatory neuropeptides: neurotensin (NT), cholecystokinin (CCK) (148–150), and vasoactive intestinal protein (151). The DA subregions also contain glial cells, including astrocytes, oligodendrocytes and microglia, which interact with neurotransmitter release (152–154). Astrocytes in particular are D2 receptor responsive, and are critical for controlling extracellular glutamate levels, which in turn alters the excitability of DA neurons (153, 155, 156).

GABA

GABAergic neurons comprise the largest nondopaminergic subpopulation among the DA subregions (157–159). While the surrounding pars reticulata is comprised entirely of GABAergic cells, inhibitory neurons are also interspersed among DA cells in all subregions (143, 160). All DA neurons appear to be regulated by GABA receptors (158). GABAergic neuron activity in the ventral midbrain can be influenced by astrocytic activity (161), in addition to afferent control by neuronal systems. GABAergic neurons establish local inhibitory connections on DAergic neurons, and also project outside the VTA to the ventral striatum, basal forebrain, the prefrontal cortex, the lateral habenula, lateral hypothalamus, and amygdala (162–167). GABAergic neurons are themselves diverse, based on morphology and immunostaining for neuropeptides, calcium-binding proteins, and nitric oxide synthase (126, 168–170).

While GABAergic neurons make up about one-third of neurons in the midbrain, the ratio of GABA-to-DA cells varies among DA subdivisions (141, 171). In nonhuman primate animals of the same age and sex (pubertal males), we found that although GABAergic neurons comprise 30% neurons, there are marked differences in the DA-to-GABAergic ratios across subpopulations, with the greatest DA-GABA ratio (5:1) in the parabrachial pigmented nucleus of the VTA, and the lowest DA-GABA ratio (1:1) in the A8 neurons. These different ratios are largely accounted for by the changes in DA neurons in each population, with GABAergic neuron numbers remaining similar across regions (140).

Colocalization of traditional markers for DA and GABA occurs in the rodent midbrain but apparently comprise a relatively small subpopulation of DA neurons (10%–15%) (172, 173). Nonetheless, GABA/DA coexpression in selective projections is recognized as an important local regulator of striatal modulation as there is an alternative GABA synthesis pathway involving aldehyde dehydroxygenase-1 (173) found in many DA neurons that may also play a role (see below, *New ways of understanding DA heterogeneity*). As will be described below, a simple "dichotomy" of DA-GABA ratios can be misleading, since many putative DA neurons that co-contain glutamate also contain glutamate aldehyde decaroxylase, GAD, an enzyme in the biosynthesis of GABA, as well as aldehyde dehydroxygenase-1. Although GAD is not detected in some studies in

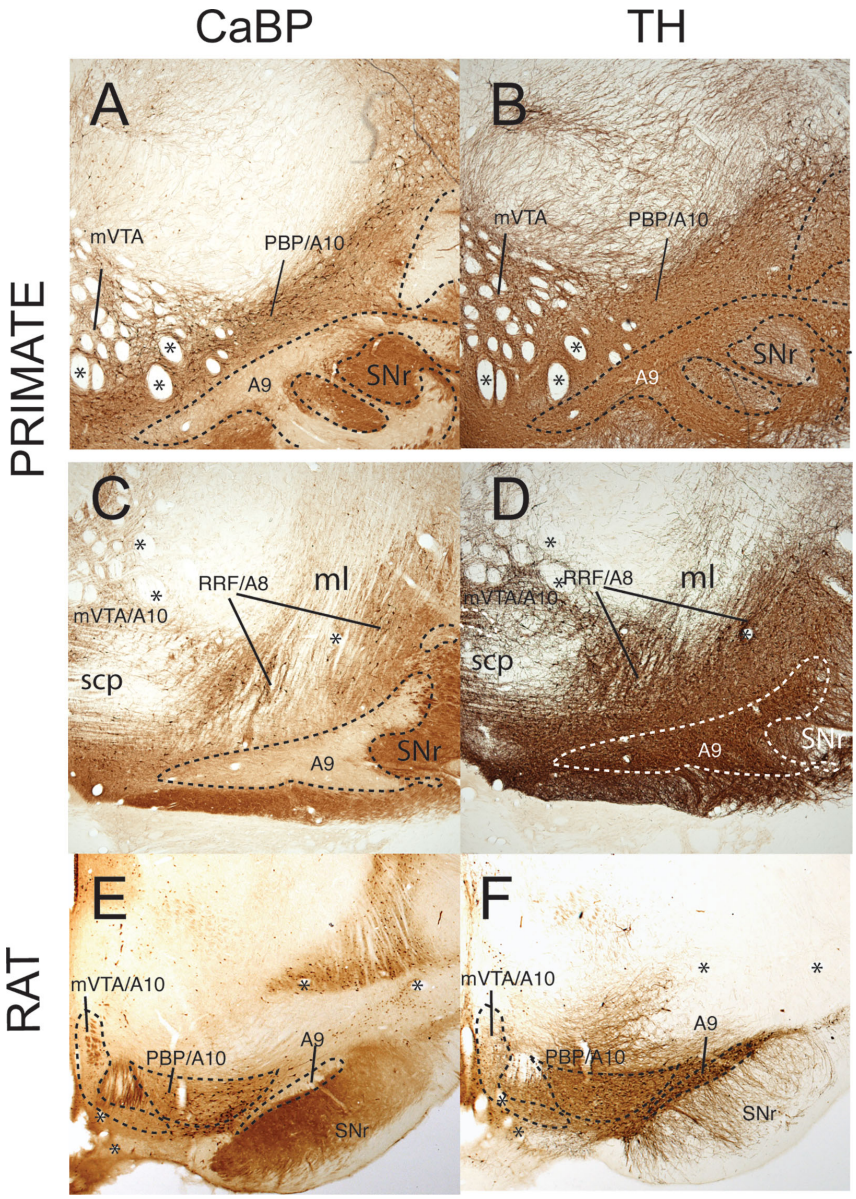

Figure 5. Localization of the A10, A9, and A8 DA subregions following immunohistochemical labeling for CaBP and TH in nonhuman primate and murine ventral midbrain. (A) Rostrocentral level of nonhuman primate ventral midbrain with CaBP-positive cells in mVTA (A10), PBP (A10) in contrast to CABP-negative labeling in A9 (dotted outline). (B) Neighboring TH-IR section to A. (C) Caudal level of nonhuman primate ventral midbrain with CaBP-positive cells in RRF/A8 in contrast to CABP-negative labeling in A9 (dotted outline). (D) Neighboring TH-IR section to C. (E) Murine ventral midbrain demonstrating CaBP-IR in VTA. A9 (substantia nigra pars compacta) is CaBP-negative. (F) Neighboring section to E showing TH-IR. Abbreviations: ml, medial lemniscus; mVTA, medial ventral tegmental area; PBP, parabrachial pigmented nucleus; RRF, retrorubral field; SNr, substantia nigra reticulata; vta, ventral tegmental area; zi, zona inserta.

mouse (174), we detected GAD1 mRNA signal both in interneurons and DA neurons in the primate.

Glutamate

The discovery that some DA neurons coexpress glutamate over twenty years ago created a paradigm shift in conceptualizing DA neuron function (142, 175). DA neurons can selectively express the glutamate vesicular transporter 2 (VGluT2), which is differentially expressed in different DA subregions (144, 176–178). Consistent with this, glutamate release from DA neurons is only detected in some forebrain targets in rodents (179).

Importantly, the corelease of DA and glutamate appears to be developmentally regulated, due to the dynamic expression of tyrosine hydroxylase (TH) (128). In early postnatal development, most postmitotic DA neuron progenitors express VGluT2 (180). These DA progenitors migrate in waves so that the earliest neurons arrive in the lateral midbrain, and the most recently differentiated neurons populate the midline structures (181, 182). During development, TH mRNA gradually increases throughout the midbrain neurons, and VGluT2 expression declines (147, 183, 184). Depending on the animal's age, the complement of VGluT2-only cells, VGluT2-TH, and TH-only neurons can theoretically shift (183) particularly in long-lived species like primates. "Pure" glutamatergic VTA neurons are largely are localized to the midline in adult mice, marmosets, and humans (185, 186), but found throughout the ventral midbrain (178).

Newer Ways of Understanding DA Heterogeneity

The understanding of the heterogeneity of DA subpopulations across the midbrain has been advanced by newer transcriptomic methods in rodents, including single-cell RNA sequencing (scRNA-seq) (187). When gene expression heterogeneity in single cells is mapped and validated spatially,

the distribution of combinations of mRNA transcripts is evident across the medial-to-lateral and dorsal/ventral extent of the system. These approaches not only confirm DA subregional "markers," also shed light on molecules not previously identified within these regions (128, 188–190). Replicating the results of traditional immunostaining studies, scRNA-seq approaches, combined with in situ hybridization, show that calcium-binding protein D28-K mRNA (CABP) maps onto the "dorsal tier" of A10 and the A8 neurons. Functionally, high CaBP levels are associated with fast buffering of calcium influx, which controls the rate of synaptic vesicle release following an action potential (191, 192). In primates CaBP + DA neurons also lack high levels of autoregulatory molecules such as the dopamine transporter (DAT) and the D2 receptor (193), suggesting a relative dependence on calcium buffering for controlling release at synapses. "High" and "low" expressing levels of these autoregulatory molecules in different DA subregions are now confirmed in scRNA-seq in mouse (194). In contrast, CaBP-negative DA neurons of the ventral substantia nigra (A9), are likely to express aldehyde dehydroxylase-1, which regulates DA production and also serves as an alternate path for GABAergic synthesis (173).

In macaques, CaBP + DA neurons neurons (A10 and A8) have specific projections to the ventral striatum, to the entire prefrontal cortex, and the amygdala (122, 138, 195–197). In mouse studies, it is found that a subset of CaBP + DA neurons further colocalize VGluT2 mRNA (121, 128, 179, 194). For example, CaBP/VGluT2-positive neurons of the midline VTA project to the ventral striatum, while the CaBP/VGluT2 of the lateral VTA(PBP), project specifically to the central nucleus of the amygdala (179) and to the tail of the caudoventral striatum (121). As with VGluT2 expression, developmental shifts in CaBP have not been fully accounted for, so that the picture as viewed from studies in adult animals may not be static through life, or applicable to younger animals (189, 190). This may be also relevant in higher species where development occurs over longer time periods. For example, in the human, CaBP gene expression in DA neurons is not seen prior to P7 (128).

Attempting to control for age and sex effects, we preliminarily examined two young male macaques for evidence of "multiplexed" transmitter content using mRNA expression for TH, VGluT2, and GAD1 (Figure 6A–H). These animals were considered "adolescent" at age 3 (early puberty) and 6 (late puberty) (198). Using RNAScope methodology and a semiautomated cell counting strategy, we found few differences between these animals in either the numbers of single-labeled or "multiplexed" neurons. Our method used RNAScope methods in evenly spaced coronal sections throughout the midbrain (5–6 sections, spaced 1 mm apart). After setting criteria for automated labeled cell detection in individual channels in the triple-labeled sections in preliminary studies, the regions of interest were drawn from adjacent CaBP-IR sections. After automated detection of each cells in individual channels, colocalization was performed (Neurolucida 360, Microbrightfield, Williston, VT) [see also (14)]. An independent investigator randomly chose sections and subregions for manual count validation, to check the fidelity of the semiautomated settings to markers placed by human expert users. We compared each animal in terms of number of cells and the relative proportion of cells counted, which was similar.

In this pilot, we were surprised to find that the majority of labeled neurons contained two or more of these three transmitters, and formed 59% of all cells (Figure 6G). The remaining 41% were single-labeled for either TH, VGluT2, or GAD1 mRNA. As expected, the majority of neurons contained TH mRNA (82%). Somewhat surprisingly, however, the majority (69%) of TH mRNA-positive neurons were multiplexed (Figure 6H), with the predominant type being TH/VGluT2-coexpressing cells (light and dark purple). A sizeable proportion of TH/VGluT2-expressing neurons also cocontained GAD1 mRNA (dark purple). Pure glutamatergic neurons (orange) were relatively rare (7%). Interestingly, GAD1 mRNA + neurons constituted about 30% of all cells, consistent with prior results for single-labeled immunohistochemical studies (140); yet these too contained sizeable "multiplexed" populations (Figure 6G). We found that GAD1 mRNA colocalization in TH-positive neurons almost always occurred against a VGluT2 background (Figure 6H). Since GAD1 protein is a synthetic enzyme responsible for converting glutamate to GABA, this tendency for TH-GAD1-VGluT2 co-localization makes anatomic sense. Past studies in rodent have not appreciated GAD1 mRNA in DA neurons, but do find corelease of GABA at the DA terminals (173, 174).

It is hard to compare this work to prior work in humans and marmosets, or rodents (185) not only due to potential species differences, but also the relative age of the individuals [human samples are elderly, when DA neurons decrease normally (183, 199)]. Importantly, a bigger sample size and the inclusion of female animals is essential to fully interpret our results. In general, however, as found previously, there are relatively fewer VGluT2-single labeled neurons in nonhuman primate, compared to rodents. In addition, there are three clear populations of TH-single, TH/VGluT2, and TH/VGluT2/GAD1 containing neurons. In contrast to rodents, and similar to one other study in marmoset and human, TH/VGluT2 containing neurons were not restricted to the midline VTA structures (185).

The relatively young age of our animals may explain the relatively high colocalization of VGluT2 mRNA in TH-positive neurons, since glutamate in dopaminergic cells is developmentally regulated as noted above [see also Fortin (147, 200)]. Furthermore, different methodologies across studies have differing sensitivity for detecting these molecules. Prior work compared immunohistochemistry for TH in conjunction with traditional methods for *in situ* double labeling of VGluT2 mRNA, whereas these results employ RNAScope to detect transcripts for all molecules.

Correlating our mRNA results with protein levels is relatively straightforward for TH mRNA and protein, and the distribution is robust and similar, as expected. The general distribution of GAD1 mRNA comported with our previous experiments for GAD1 protein, as noted above. VGluT2 protein is found at terminals, although its transcript is found in the cell body, leading to a mismatch of protein and transcript localization (201). The presence of VGluT2 mRNA at the cell body, however, has been found to be necessary and sufficient for glutamate exocytosis at the synapse, and is considered a marker of glutamatergic transmisson (202).

Mapping CRF Receptors onto a Heterogeneous DA System

A comprehensive understanding of CRF effects in the DA system requires a thorough understanding of CRF receptors distribution among specific cell types and circuits in the DA system. In older anatomic studies, CRFR1 mRNA is distributed over the VTA and other midbrain structures (64, 203, 204), whereas there is reportedly little CRFR2 mRNA (204). One important issue with lower resolution anatomic studies is that CRFR2 mRNA and protein are found in axon terminals innervating the ventral midbrain (70, 97). Therefore, if CRFR2 mRNA (or protein) is not properly colocalized in presynaptic or postsynaptic structures using appropriate markers, the data can be hard to interpret (67, 203).

More recent studies using mouse genetics indicate that CRFR1 is found with relative abundance in DA versus non-DA cells (100, 103, 205), consistent with patch clamp studies showing the CRFR1 activates DA neurons, increasing spontaneous firing (88). CRFR1-containing GABAergic neurons exist, but do not change their spontaneous firing pattern in response to CRF (103). A recent report in mouse indicates that CRFR1 + neurons in the VTA are > 87% DAergic, and segregate to the lateral VTA (PBP) neurons which project to the lateral shell and core, but not the medial shell, of the ventral striatum (205). While data in monkey are preliminary, we find a very widespread distribution of CRFR1 mRNA in the ventral midbrain, mainly localized to DA neurons. CRFR1 is distributed broadly, not only in the A10 neurons but also in the substantia nigra, pars compacta (SNc) (Figure 6I). As in mouse, the lateral VTA (PBP) cells appear more enriched in CRFR1 mRNA than the midline VTA cells, and CRFR1 mRNA is strongly expressed across the SNc DA neurons as well. In adolescent animals, we found that on average, 72% of CRFR1 mRNA-positive neurons across all ventral midbrain subregions contained TH mRNA. Most of the CRFR1-TH–positive neurons were "multiplexed" with GAD1 and/or VGluT2 (not shown) (Figure 6J).

Less is understood about the anatomy of the CRFR2 distribution in the midbrain. Several reports using real-time PCR found low-level CRFR2

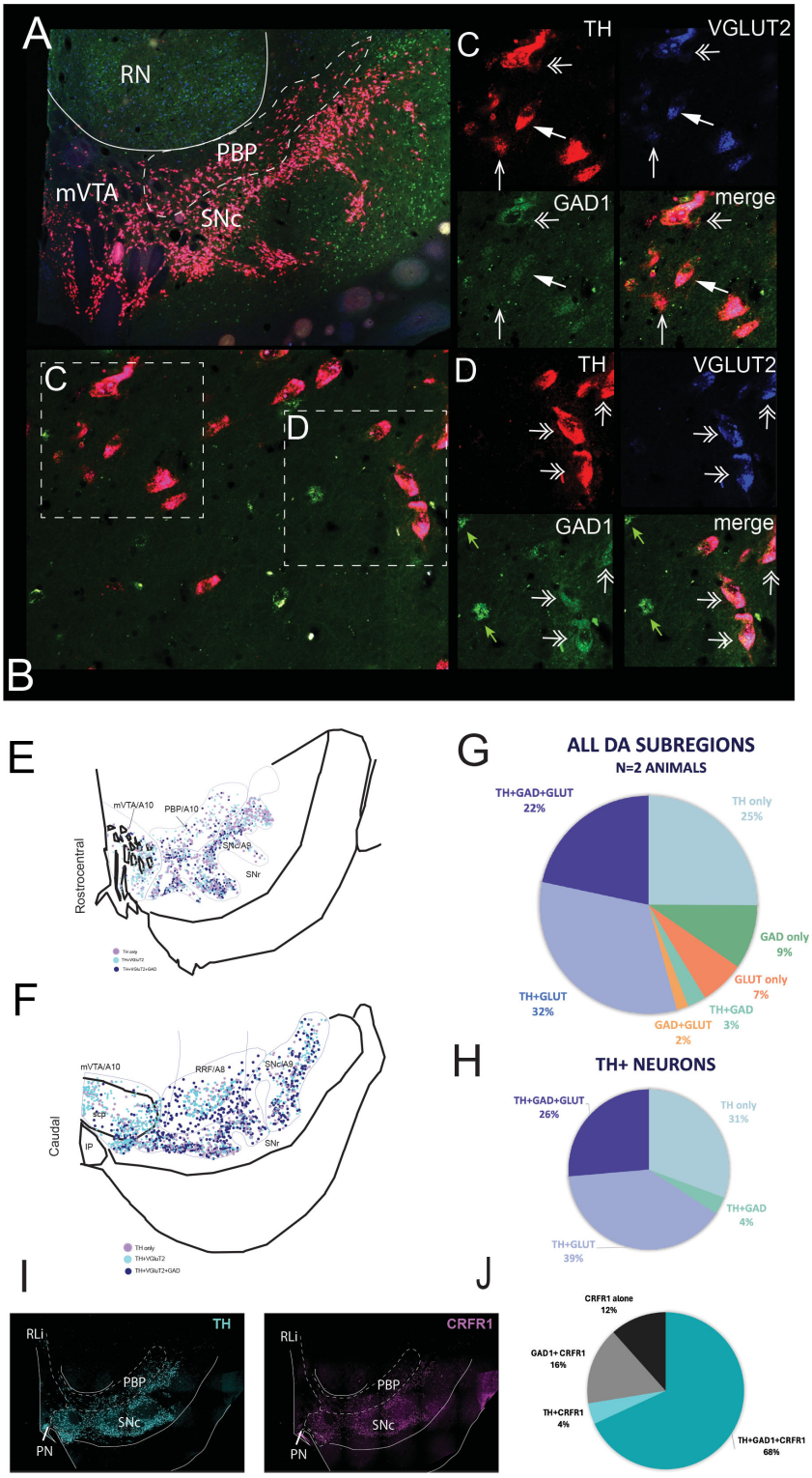

Figure 6. Preliminary characterization of "multiplexed" neurons in A10, A9, and A8 subregions in macaque. (A) Overview of RNAScope processed section at 4X, (B–D) Higher power (20X) images of various neuronal types in separate and merged channels. (C) Insets with TH mRNA + neurons in red, either single labeled, or colabeled with VGluT2 (blue) and/or GAD1 (green) mRNA. Simple arrow = single-labeled TH + neurons, arrowhead = TH/VGluT2 + neuron, double arrowhead = triple-labeled TH/VGluT2/GAD1 neurons (not all cells are labeled for simplicity). (D) Insets with similar neurons types, but depicting many triple-labeled neurons (double arrowhead), and single labeled GAD1 mRNA + neurons (green arrows). (E, F) Maps of the distribution of TH-positive phenotypes at two rostrocaudal levels. Light blue, TH only; light purple, TH/vGLUT2; dark purple, TH/vGLUT2/GAD1. (G) Mulitplexed and nonmultiplexed neurons in the ventral midbrain. (H) Proportions of multiplexed neuronal types by transmitter types. (I) Macroscopic view of CRFR1 mRNA in macaque ventral midbrain. The majority of CRFR1 mRNA is colocalized in TH-positive neurons (n = 3 animals). (J) Triple labeling for TH-GAD1-CRFR1 mRNA shows that the majority of CRFR1 neurons cocontain TH mRNA.

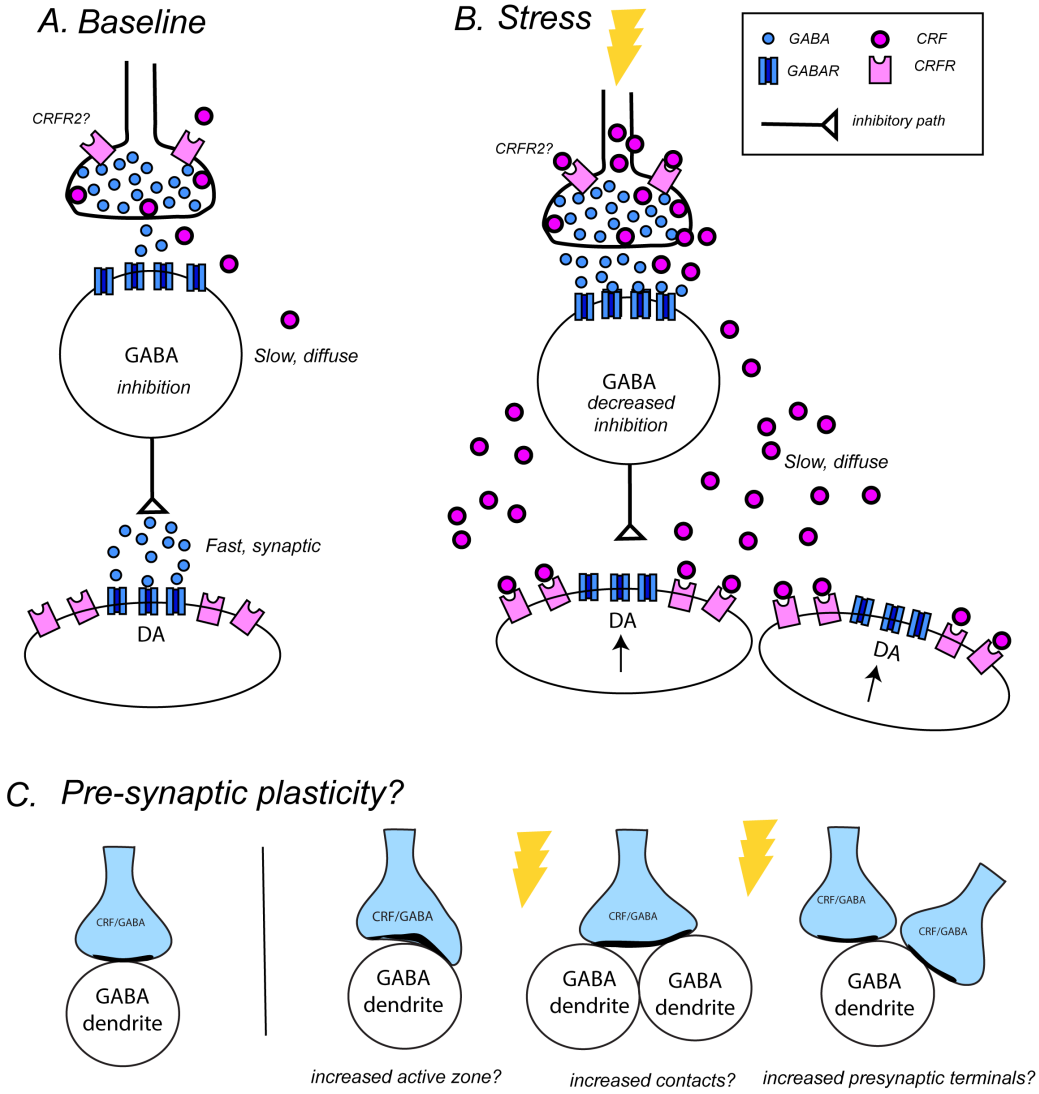

Figure 7. Piecing together CRF-DA anatomic relationships. (A) CRF can act via dual "fast"/short range and "slow"/long range mechanisms. CRF/ GABA afferent terminals mainly contact non-DA neurons (putative interneurons). GABA-CRF terminals may autoregulate GABA release through presynaptic CRF2 receptors, as in other brain regions. In the midbrain, classic GABAergic transmission "inhibits" the inhibitory GABAergic neuron, to modulate DA firing. (B) Under stress, increased activity-dependent CRF production acts via both increased presynaptic transmission (increasing GABAergic fast signaling, blue) and volume (slow, pink) transmission. Volume transmission permits CRF to more completely saturate CRFR1 receptors on DA membranes at some distance from extrasynaptic release sites. In this way, it works in tandem with GABAergic signaling, ensuring that interneuron inhibition is decreased, before direct excitation via CRF1R signaling. (C) Stress and CRF can change synaptic structure to enhance CRF effects (see text), although this possibility has not been explored in the CRF-DA path. Increased active zone length and increased contact sites are potential mechanisms.

expression in the mouse VTA; however, these results have not been reproduced using in situ hybridization until recently.

While one report using real-time PCR found low-level CRFR2 expression in the mouse VTA (101), other researchers were unable to reproduce those results (34, 113). Recently, using modern methods for in situ hybridization, low baseline levels of CRFR2 were found in astrocytes (206). Interestingly, CRFR2 was significantly upregulated by dopaminergic agonists (cocaine and methamphetamine), and was seen in astrocytes and TH-positive neurons after drug treatment. These studies suggest that low baseline levels of CRFR2 in the midbrain are up-regulated by manipulations such as stress or substances (86, 206), similar to effects on CRFR1 in diverse brain regions.

Future Directions

In summary, the mapping of gene expression patterns within distinct DA subpopulations highlights a nuanced spatial distribution of TH, GAD1, and VGluT2 mRNA transcripts that may change dynamically with age (182). We are attempting to add in the distribution of CRF and CRFR1 the macaque. We previously found that the vast majority CRF + axon terminals in this region make contacts onto non-DA (presumptive GABAergic) neurons (33). CRFR1 mRNA is broadly distributed on DA neurons in the lateral VTA and in the substantia nigra (A9), but it is unclear how development, sex differences, and stress factor into this pattern. This first "snapshot" suggests that at least in young male animals, control of GABAergic neurons by CRF in the lateral VTA is through direct synaptic, possibly presynaptic, mechanisms, while strong labeling for CRFR1 mRNA in DA neurons (and relatively few direct CRF + synapses onto these cells), suggests that CRF "volume" transmission is also in play (Figure 7A and B).

"Volume transmission," long described in seminal studies by Agnati, Fuxe, and colleagues (207), explains how peptides confer long-range and enduring effects on signaling, which are complementary to excitatory, "fast" transmission. Peptides such as CRF "packaged" with classic fast

transmitters in the same neuron allows for cooperativity and flexibility at the target. They are packaged in large dense core vesicles, which require high-frequency action potentials for release, in contrast to classic transmitters. Once released, peptides such as CRF may take seconds to minutes to reach their receptors which are often found some distance from the synapse, in contrast to synaptic fast transmission that occurs within milliseconds through a synaptic cleft of less than 50 nm (208, 209). Neuropeptide clearance is slower, unlike classic transmitter reuptake, and depends on extracellular peptidases. These dual mechanisms provide variations in spatial and temporal control of DA firing (37, 68, 210). In a simple model suggested by our preliminary results, GABA release can quickly release the inhibitory (GABA interneuron) brakes on DA, while more intense firing rates in CRF/GABA terminals activates release of extrasynaptic CRF which diffuses through the extracellular space to directly activate CRFR1 in a concentration dependent manner (Figure 7A and B). On the postsynaptic side, CRFR1 receptors on DA neurons may form oligomeric complexes with other G protein–coupled receptors on the membrane surface, to integrate peptide signaling (211).

Beyond signaling dynamics, another relatively underexplored facet of the field is that CRF release has neuroplastic effects on synapse structure (212–215). This is a potentially important modality for conferring long-lasting synaptic changes in the stress system, including DA release (Figure 7C). Determining neuroplastic effects of CRF-containing projections on the DA system will require exhaustive work using laborious techniques like electron microscopy, but will be important on shedding light on how long-term adaptations in the stress system occur, particularly in higher species.

Authors Contributions

J.L.F. supervised I.M. on literature review, and both wrote the manuscript; J.L.F. and E.A.K. provided editing and selection of figures. I.M. and E.A.K collected and analyzed preliminary, original data presented in the manuscript. E.A.K. contacted authors of adapted figures for permission to present their work in the literature review. All authors take full responsibility for all data, figures, and text and approve the content and submission of the study, supervised by J.L.F. No related work is under onsideration elsewhere. All authors state that all unprocessed data are available, and all authors read and approved the paper.

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Author Disclosures

The authors have no competing interests to declare.

References

1. Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science. 1981;213(4514):1394–7. DOI: 10.1126/science.6267699. PMID: 6267699
2. Kelly EA, Fudge JL. The neuroanatomic complexity of the CRF and DA systems and their interface: what we still don't know. Neurosci Biobehav Rev. 2018;90:247–59. DOI: 10.1016/j.neubiorev.2018.04.014. PMID: 29704516; PMCID: PMC5993645
3. Pawlikowski M, Zelazowski P, Dohler K, Stepien H. Effects of two neuropeptides, somatoliberin (GRF) and corticoliberin (CRF), on human lymphocyte natural killer activity. Brain Behav Immun. 1988;2(1):50–6. DOI: 10.1016/0889-1591(88)90005-0. PMID: 3140923
4. Wypior G, Jeschke U, Kurpisz M, Szekeres-Bartho J.Expression of CRH, CRH-related peptide and CRH receptor in the ovary and potential CRH signalling pathways. J Reprod Immunol. 2011;90(1):67–73. DOI: 10.1016/j.jri.2011.04.009. PMID: 21696829
5. Huising MO, van der Meulen T, Vaughan JM, Matsumoto M, Donaldson CJ, Park H, et al. CRFR1 is expressed on pancreatic beta cells, promotes beta cell proliferation, and potentiates insulin secretion in a glucose-dependent manner. Proc Natl Acad Sci U S A. 2010;107(2):912–7. DOI: 10.1073/pnas.0913610107. PMID: 20080775; PMCID: PMC2818901
6. Takahashi K. Distribution of urocortins and corticotropin-releasing factor receptors in the cardiovascular system. Int J Endocrinol. 2012;2012:395284. DOI: 10.1155/2012/395284. PMID: 22675352; PMCID: PMC3362921
7. Hiroi N, Wong ML, Licinio J, Park C, Young M, Gold PW, et al. Expression of corticotropin releasing hormone receptors type I and type II mRNA in suicide victims and controls. Mol Psychiatry. 2001;6(5):540–6. DOI: 10.1038/sj.mp.4000908. PMID: 11526468
8. Heim C, Shugart M, Craighead WE, Nemeroff CB. Neurobiological and psychiatric consequences of child abuse and neglect. Dev Psychobiol. 2010;52(7):671–90. DOI: 10.1002/dev.20494. PMID: 20882586
9. Carrasco GA, Van de Kar LD. Neuroendocrine pharmacology of stress. Eur J Pharmacol. 2003;463(1-3):235–72. DOI: 10.1016/s0014-2999(03)01285-8. PMID: 12600714
10. Ressler KJ, Berretta S, Bolshakov VY, Rosso IM, Meloni EG, Rauch SL, et al. Post-traumatic stress disorder: clinical and translational neuroscience from cells to circuits. Nat Rev Neurol. 2022;18(5):273–88. DOI: 10.1038/s41582-022-00635-8. PMID: 35352034; PMCID: PMC9682920
11. Howes OD, McCutcheon R, Owen MJ, Murray RM. The role of genes, stress, and dopamine in the development of schizophrenia. Biol Psychiatry. 2017;81(1):9–20. DOI: 10.1016/j.biopsych.2016.07.014. PMID: 27720198; PMCID: PMC5675052
12. Swanson LW, Sawchenko PE, Rivier J, Vale WW. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology. 1983;36(3):165–86. DOI: 10.1159/000123454. PMID: 6601247
13. Olschowka JA, O'Donohue TL, Mueller GP, Jacobowitz DM. The distribution of corticotropin releasing factor-like immunoreactive neurons in rat brain. Peptides. 1982;3(6):995–1015. DOI: 10.1016/0196-9781(82)90071-7. PMID: 6984756
14. Fudge JL, Kelly EA, Hackett TA. Corticotropin releasing factor (CRF) co-expression in GABAergic, glutamatergic and GABA/glutamatergic subpopulations in the central extended amygdala and ventral pallidum of young male primates. J Neurosci. 2022;42:8997–9010. DOI: 10.1523/JNEUROSCI.1453-22.2022. PMID: 36280261; PMCID: PMC9732834
15. Bassett JL, Foote SL. Distribution of corticotropin-releasing factor-like immunoreactivity in squirrel monkey (Saimiri sciureus) amygdala. J Comp Neurol. 1992;323(1):91–102. DOI: 10.1002/cne.903230108. PMID: 1430317
16. Paull WK, Phelix CF, Copeland M, Palmiter P, Gibbs FP, Middleton C. Immunohistochemical localization of corticotropin releasing factor (CRF) in the hypothalamus of the squirrel monkey, Saimiri sciureus. Peptides. 1984;5 Suppl 1:45–51. DOI: 10.1016/0196-9781(84)90264-x. PMID: 6384953
17. Alheid GF. Extended amygdala and basal forebrain. Ann N Y Acad Sci. 2003;985:185–205. DOI: 10.1111/j.1749-6632.2003.tb07082.x. PMID: 12724159
18. Heimer L, De Olmos JS, Alheid GF, Person J, Sakamoto N, Shinoda K, et al. The human basal forebrain. Part II. In: Bloom FE, Bjorkland A, Hokfelt T, editors. Handbook of Chemical Neuroanatomy. 15: The Primate Nervous System, Part III. Amsterdam: Elsevier; 1999. p. 57–226.
19. Rodaros D, Caruana DA, Amir S, Stewart J. Corticotropin-releasing factor projections from limbic forebrain and paraventricular nucleus of the hypothalamus to the region of the ventral tegmental area. Neuroscience. 2007;150(1):8–13. DOI: 10.1016/j.neuroscience.2007.09.043. PMID: 17961928
20. Dabrowska J, Martinon D, Moaddab M, Rainnie DG. Targeting corticotropin-releasing factor (CRF) projections from the oval nucleus of the BNST using cell-type specific neuronal tracing studies in mouse and rat brain. J Neuroendocrinol. 2016;28:10.1111/jne.12442. DOI: 10.1111/jne.12442. PMID: 27805752; PMCID: PMC5362295
21. Fudge JL, Kelly EA, Pal R, Bedont JL, Park L, Ho B. Beyond the classic VTA: extended amygdala projections to DA-striatal paths in the primate. Neuropsychopharmacology. 2017;42(8):1563–76. DOI: 10.1038/npp.2017.38. PMID: 28220796; PMCID: PMC5518904
22. Thierry AM, Tassin JP, Blanc G, Glowinski J. Selective activation of mesocortical DA system by stress. Nature. 1976;263(5574):242–4. DOI: 10.1038/263242a0. PMID: 958479
23. Abercrombie ED, Keefe KA, DiFrischia DS, Zigmond MJ. Differential effects of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. J Neurochem. 1989;52:1655–8. DOI: 10.1111/j.1471-4159.1989.tb09224.x. PMID: 2709017
24. Coco ML, Kuhn CM, Ely TD, Kilts CD. Selective activation of mesoamygdaloid dopamine neurons by conditioned stress: attenuation by diazepam. Brain Res. 1992;590(1-2):39–47. DOI: 10.1016/0006-8993(92)91079-t. PMID: 1422845
25. Kilts CD, Coco ML, Ely TD, Bissette G, Nemeroff CB. Differential effects of conditioned and unconditioned stress on the neurotensin content of dopamine cell body groups of the ventral mesencephalon. Ann NY Acad Sci. 1992;668:266–76. DOI: 10.1111/j.1749-6632.1992.tb27355.x. PMID: 1361117
26. Inglis FM, Moghaddam B. Dopaminergic innervation of the amygdala is highly responsive to stress. J Neurochem. 1999;72(3):1088–94. DOI: 10.1046/j.1471-4159.1999.0721088.x. PMID: 10037480

27. Menegas W, Akiti K, Amo R, Uchida N, Watabe-Uchida M. Dopamine neurons projecting to the posterior striatum reinforce avoidance of threatening stimuli. Nat Neurosci. 2018;21(10):1421–30. DOI: 10.1038/s41593-018-0222-1. PMID: 30177795; PMCID: PMC6160326
28. Soden ME, Yee JX, Cuevas B, Rastani A, Elum J, Zweifel LS. Distinct encoding of reward and aversion by peptidergic BNST inputs to the VTA. Front Neural Circuits. 2022;16:918839. DOI: 10.3389/fncir.2022.918839. PMID: 35860212; PMCID: PMC9289195
29. Chang S, Fermani F, Lao CL, Huang L, Jakovcevski M, Di Giaimo R, et al. Tripartite extended amygdala-basal ganglia CRH circuit drives locomotor activation and avoidance behavior. Sci Adv. 2022;8(46):eabo1023. DOI: 10.1126/sciadv.abo1023. PMID: 36383658; PMCID: PMC9668302
30. Hupalo S, Bryce CA, Bangasser DA, Berridge CW, Valentino RJ, Floresco SB. Corticotropin-releasing factor (CRF) circuit modulation of cognition and motivation. Neurosci Biobehav Rev. 2019;103:50–9. DOI: 10.1016/j.neubiorev.2019.06.010. PMID: 31212019; PMCID: PMC6692202
31. Sanders J, Nemeroff C. The CRF System as a therapeutic target for neuropsychiatric disorders. Trends Pharmacol Sci. 2016;37:1045–54. DOI: 10.1016/j.tips.2016.09.004. PMID: 27717506; PMCID: PMC5121012
32. Foote SL, Cha CI. Distribution of corticotropin-releasing-factor-like immunoreactivity in brainstem of two monkey species (Saimiri sciureus and Macaca fascicularis): an immunohistochemical study. J Comp Neurol. 1988;276(2):239–64. DOI: 10.1002/cne.902760208. PMID: 3265422
33. Kelly EA, Love TM, Fudge JL. Corticotropin-releasing factor-dopamine interactions in male and female macaque: beyond the classic VTA. Synapse. 2023:78(1):e22284. DOI: 10.1002/syn.22284. PMID: 37996987; PMCID: PMC10842953
34. Tagliaferro P, Morales M. Synapses between corticotropin-releasing factor-containing axon terminals and dopaminergic neurons in the ventral tegmental area are predominantly glutamatergic. J Comp Neurol. 2008;506(4):616–26. DOI: 10.1002/cne.21576. PMID: 18067140; PMCID: PMC2440343
35. Halliday GM, Tork I. Comparative anatomy of the ventromedial mesencephalic tegmentum in the rat, cat, monkey and human. J Comp Neurol. 1986;252:423–45. DOI: 10.1002/cne.902520402. PMID: 3782510
36. Francois C, Yelnik J, Tande D, Agid Y, Hirsch EC. Dopaminergic cell group A8 in the monkey: anatomical organization and projections to the striatum. J Comp Neurol. 1999;414(3):334–47. PMID: 10516600
37. Nusbaum MP, Blitz DM, Marder E. Functional consequences of neuropeptide and small-molecule co-transmission. Nat Rev Neurosci. 2017;18(7):389–403. DOI: 10.1038/nrn.2017.56. PMID: 28592905; PMCID: PMC5547741
38. Hokfelt T, Bartfai T, Bloom F. Neuropeptides: opportunities for drug discovery. Lancet Neurol. 2003;2(8):463–72. DOI: 10.1016/s1474-4422(03)00482-4. PMID: 12878434
39. Gallagher JP, Orozco-Cabal LF, Liu J, Shinnick-Gallagher P. Synaptic physiology of central CRH system. Eur J Pharmacol. 2008;583(2-3):215–25. DOI: 10.1016/j.ejphar.2007.11.075. PMID: 18342852; PMCID: PMC2424315
40. Pomrenze MB, Giovanetti SM, Maiya R, Gordon AG, Kreeger LJ, Messing RO. Dissecting the roles of GABA and neuropeptides from rat Central amygdala CRF neurons in anxiety and fear learning. Cell Rep. 2019;29(1):13–21.e4. DOI: 10.1016/j.celrep.2019.08.083. PMID: 31577943; PMCID: PMC6879108
41. Chen Y, Andres AL, Frotscher M, Baram TZ. Tuning synaptic transmission in the hippocampus by stress: the CRH system. Front Cell Neurosci. 2012;6:13. DOI: 10.3389/fncel.2012.00013. PMID: 22514519; PMCID: PMC3322336
42. Thureson-Klein AK, Klein RL. Exocytosis from neuronal large dense-cored vesicles. Int Rev Cytol. 1990;121:67–126. DOI: 10.1016/s0074-7696(08)60659-2. PMID: 1972143
43. Henckens MJ, Deussing JM, Chen A. Region-specific roles of the corticotropin-releasing factor-urocortin system in stress. Nat Rev Neurosci. 2016;17(10):636–51. DOI: 10.1038/nrn.2016.94. PMID: 27586075
44. Dautzenberg FM, Hauger RL. The CRF peptide family and their receptors: yet more partners discovered. Trends Pharmacol Sci. 2002;23(2):71–7. DOI: 10.1016/s0165-6147(02)01946-6. PMID: 11830263
45. Lovenberg TW, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, De Souza EB, et al. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. Proc Natl Acad Sci U S A. 1995;92(3):836–40. DOI: 10.1073/pnas.92.3.836. PMID: 7846062; PMCID: PMC42715
46. Perrin MH, Vale WW. Corticotropin releasing factor receptors and their ligand family. Ann N Y Acad Sci. 1999;885:312–28. DOI: 10.1111/j.1749-6632.1999.tb08687.x. PMID: 10816663
47. Ma S, Shen Q, Zhao LH, Mao C, Zhou XE, Shen DD, et al. Molecular basis for hormone recognition and activation of corticotropin-releasing factor receptors. Mol Cell. 2020;77(3):669–80.e4. DOI: 10.1016/j.molcel.2020.01.013. PMID: 32004470
48. Vaughan J, Donaldson C, Bittencourt J, Perrin MH, Lewis K, Sutton S, et al. Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. Nature. 1995;378(6554):287–92. DOI: 10.1038/378287a0. PMID: 7477349
49. Reyes TM, Lewis K, Perrin MH, Kunitake KS, Vaughan J, Arias CA, et al. Urocortin II: a member of the corticotropin-releasing factor (CRF) neuropeptide family that is selectively bound by type 2 CRF receptors. Proc Natl Acad Sci U S A. 2001;98(5):2843–8. DOI: 10.1073/pnas.051626398. PMID: 11226328; PMCID: PMC30227
50. Lewis K, Li C, Perrin MH, Blount A, Kunitake K, Donaldson C, et al. Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. Proc Natl Acad Sci U S A. 2001;98(13):7570–5. DOI: 10.1073/pnas.121165198. PMID: 11416224; PMCID: PMC34709
51. Bale TL, Contarino A, Smith GW, Chan R, Gold LH, Sawchenko PE, et al. Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. Nat Genet. 2000;24(4):410–4. DOI: 10.1038/74263. PMID: 10742108
52. Kishimoto T, Radulovic J, Radulovic M, Lin CR, Schrick C, Hooshmand F, et al. Deletion of crhr2 reveals an anxiolytic role for corticotropin-releasing hormone receptor-2. Nat Genet. 2000;24(4):415–9. DOI: 10.1038/74271. PMID: 10742109
53. Radulovic J, Ruhmann A, Liepold T, Spiess J. Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: differential roles of CRF receptors 1 and 2. Journal Neurosci. 1999;19(12):5016–25. DOI: 10.1523/JNEUROSCI.19-12-05016.1999. PMID: 10366634; PMCID: PMC6782638
54. Zorrilla EP, Valdez GR, Nozulak J, Koob GF, Markou A. Effects of antalarmin, a CRF type 1 receptor antagonist, on anxiety-like behavior and motor activation in the rat. Brain Res. 2002;952(2):188–99. DOI: 10.1016/s0006-8993(02)03189-x. PMID: 12376179
55. Muller MB, Zimmermann S, Sillaber I, Hagemeyer TP, Deussing JM, Timpl P, et al. Limbic corticotropin-releasing hormone receptor 1 mediates anxiety-related behavior and hormonal adaptation to stress. Nat Neurosci. 2003;6(10):1100–7. DOI: 10.1038/nn1123. PMID: 12973355
56. Smith GW, Aubry JM, Dellu F, Contarino A, Bilezikjian LM, Gold LH, et al. Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. Neuron. 1998;20(6):1093–102. DOI: 10.1016/s0896-6273(00)80491-2. PMID: 9655498
57. Janssen D, Kozicz T. Is it really a matter of simple dualism? Corticotropin-releasing factor receptors in body and mental health. Front Endocrinol (Lausanne). 2013;4:28. DOI: 10.3389/fendo.2013.00028. PMID: 23487366; PMCID: PMC3594922
58. Sztainberg Y, Kuperman Y, Tsoory M, Lebow M, Chen A. The anxiolytic effect of environmental enrichment is mediated via amygdalar CRF receptor type 1. Mol Psychiatry. 2010;15(9):905–17. DOI: 10.1038/mp.2009.151. PMID: 20084060
59. Sztainberg Y, Kuperman Y, Justice N, Chen A. An anxiolytic role for CRF receptor type 1 in the globus pallidus. J Neurosci. 2011;31(48):17416–24. DOI: 10.1523/JNEUROSCI.3087-11.2011. PMID: 22131403; PMCID: PMC6623832
60. Millan MA, Jacobowitz DM, Hauger RL, Catt KJ, Aguilera G. Distribution of corticotropin-releasing factor receptors in primate brain. Proc Natl Acad Sci U S A. 1986;83(6):1921–5. DOI: 10.1073/pnas.83.6.1921. PMID: 2869491; PMCID: PMC323196
61. Grigoriadis DE, Dent GW, Turner JG, Uno H, Shelton SE, De Souza EB, et al. Corticotropin-releasing factor (CRF) receptors in infant rhesus monkey brain and pituitary gland: biochemical characterization and autoradiographic localization. Dev Neurosci. 1995;17(5-6):357–67. DOI: 10.1159/000111306. PMID: 8829925
62. Sanchez MM, Young LJ, Plotsky PM, Insel TR. Autoradiographic and in situ hybridization localization of corticotropin-releasing factor 1 and 2 receptors in nonhuman primate brain. J Comp Neurol. 1999;408(3):365–77. PMID: 10340512
63. Kostich WA, Grzanna R, Lu NZ, Largent BL. Immunohistochemical visualization of corticotropin-releasing factor type 1 (CRF1) receptors in monkey brain. J Comp Neurol. 2004;478(2):111–25. DOI: 10.1002/cne.20271. PMID: 15349973
64. Van Pett K, Viau V, Bittencourt JC, Chan RK, Li HY, Arias C, et al. Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. J Comp Neurol. 2000;428(2):191–212. DOI: 10.1002/1096-9861(20001211)428:2⟨191::aid-cne1⟩3.0.co;2-u. PMID: 11064361
65. Habib KE, Weld KP, Rice KC, Pushkas J, Champoux M, Listwak S, et al. Oral administration of a corticotropin-releasing hormone receptor antagonist significantly attenuates behavioral, neuroendocrine, and autonomic responses to stress in primates. Proc Natl Acad Sci U S A. 2000;97(11):6079–84. DOI: 10.1073/pnas.97.11.6079. PMID: 10823952; PMCID: PMC18561

66. Zobel AW, Nickel T, Kunzel HE, Ackl N, Sonntag A, Ising M, et al. Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. J Psychiatr Res. 2000;34(3):171–81. DOI: 10.1016/s0022-3956(00)00016-9. PMID: 10867111
67. Treweek JB, Jaferi A, Colago EE, Zhou P, Pickel VM. Electron microscopic localization of corticotropin-releasing factor (CRF) and CRF receptor in rat and mouse central nucleus of the amygdala. J Comp Neurol. 2009;512(3):323–35. DOI: 10.1002/cne.21884. PMID: 19003957; PMCID: PMC2873768
68. Reyes BAS, Bangasser DA, Valentino RJ, Van Bockstaele EJ. Using high resolution imaging to determine trafficking of corticotropin-releasing factor receptors in noradrenergic neurons of the rat locus coeruleus. Life Sci. 2014; 112(1-2):2–9. DOI: 10.1016/j.lfs.2014.07.017. PMID: 25058917; PMCID: PMC4163082
69. Waselus M, Nazzaro C, Valentino RJ, Van Bockstaele EJ. Stress-induced redistribution of corticotropin-releasing factor receptor subtypes in the dorsal raphe nucleus. Biol Psychiatry. 2009;66(1):76–83. DOI: 10.1016/j.biopsych.2009.02.014. PMID: 19362706; PMCID: PMC2728006
70. Slater PG, Noches V, Gysling K. Corticotropin-releasing factor type-2 receptor and corticotropin-releasing factor-binding protein coexist in rat ventral tegmental area nerve terminals originated in the lateral hypothalamic area. Eur J Neurosci. 2016;43(2):220–9. DOI: 10.1111/ejn.13113. PMID: 26503565
71. Tian JB, Shan X, Bishop GA, King JS. Presynaptic localization of a truncated isoform of the type 2 corticotropin releasing factor receptor in the cerebellum. Neuroscience. 2006;138(2):691–702. DOI: 10.1016/j.neuroscience.2005.11.052. PMID: 16413121
72. Zhu M, Rogers NG, Jahad JV, Herman MA. Sex differences in the impact of electronic nicotine vapor on corticotropin-releasing factor receptor 1 neurons in the mouse ventral tegmental area. J Neurosci. 2023;43(17):3081–93. DOI: 10.1523/JNEUROSCI.2087-22.2023. PMID: 37001989; PMCID: PMC10146490
73. Bangasser DA, Curtis A, Reyes BA, Bethea TT, Parastatidis I, Ischiropoulos H, et al. Sex differences in corticotropin-releasing factor receptor signaling and trafficking: potential role in female vulnerability to stress-related psychopathology. Mol Psychiatry. 2010;15(9):877, 96–904. DOI: 10.1038/mp.2010.66. PMID: 20548297; PMCID: PMC2935505
74. McAlinn HR, Reich B, Contoreggi NH, Kamakura RP, Dyer AG, McEwen BS, et al. Sex differences in the subcellular distribution of corticotropin-releasing factor receptor 1 in the rat hippocampus following chronic immobilization stress. Neuroscience. 2018;383:98–113. DOI: 10.1016/j.neuroscience.2018.05.007. PMID: 29753863; PMCID: PMC5994383
75. Enman NM, Reyes BAS, Shi Y, Valentino RJ, Van Bockstaele EJ. Sex differences in morphine-induced trafficking of mu-opioid and corticotropin-releasing factor receptors in locus coeruleus neurons. Brain Res. 2019;1706:75–85. DOI: 10.1016/j.brainres.2018.11.001. PMID: 30391476
76. Deussing JM, Chen A. The corticotropin-releasing factor Family: physiology of the stress response. Physiol Rev. 2018;98(4):2225–86. DOI: 10.1152/physrev.00042.2017. PMID: 30109816
77. Amin M, Horst N, Wu R, Gragnoli C. Novel corticotropin-releasing hormone receptor genes (CRHR1 and CRHR2) linkage to and association with polycystic ovary syndrome. J Ovarian Res. 2023;16(1):155. DOI: 10.1186/s13048-023-01159-5. PMID: 37543650; PMCID: PMC10403835
78. Binder EB, Nemeroff CB. The CRF system, stress, depression and anxiety-insights from human genetic studies. Mol Psychiatry. 2010;15(6):574–88. DOI: 10.1038/mp.2009.141. PMID: 20010888; PMCID: PMC3666571
79. Tache Y, Larauche M, Yuan PQ, Million M. Brain and gut CRF signaling: biological actions and role in the gastrointestinal tract. Curr Mol Pharmacol. 2018;11(1):51–71. DOI: 10.2174/1874467210666170224095741. PMID: 28240194; PMCID: PMC8091865
80. Risch N, Merikangas K. The future of genetic studies of complex human diseases. Science. 1996;273(5281):1516–7. DOI: 10.1126/science.273.5281.1516. PMID: 8801636
81. Lee PH, Anttila V, Won HJ, Feng YCA, Rosenthal J, Zhu ZZ, et al. Genomic relationships, novel loci, and pleiotropic mechanisms across eight psychiatric disorders. Cell. 2019;179(7):1469–82. DOI: 10.1016/j.cell.2019.11.020. PMID: 31835028; PMCID: PMC7077032
82. Koolhaas JM, Bartolomucci A, Buwalda B, de Boer SF, Flugge G, Korte SM, et al. Stress revisited: a critical evaluation of the stress concept. Neurosci Biobehav Rev. 2011;35(5):1291–301. DOI: 10.1016/j.neubiorev.2011.02.003. PMID: 21316391
83. Zweifel LS, Fadok JP, Argilli E, Garelick MG, Jones GL, Dickerson TM, et al. Activation of dopamine neurons is critical for aversive conditioning and prevention of generalized anxiety. Nat Neurosci. 2011;14(5):620–6. DOI: 10.1038/nn.2808. PMID: 21499253; PMCID: PMC3083461
84. Brischoux F, Chakraborty S, Brierley DI, Ungless MA. Phasic excitation of dopamine neurons in ventral VTA by noxious stimuli. Proc Natl Acad Sci U S A. 2009;106(12):4894–9. DOI: 10.1073/pnas.0811507106. PMID: 19261850; PMCID: PMC2660746
85. Merali Z, Lacosta S, Anisman H. Effects of interleukin-1beta and mild stress on alterations of norepinephrine, dopamine and serotonin neurotransmission: a regional microdialysis study. Brain Res. 1997;761(2):225–35. DOI: 10.1016/s0006-8993(97)00312-0. PMID: 9252020
86. Holly EN, DeBold JF, Miczek KA. Increased mesocorticolimbic dopamine during acute and repeated social defeat stress: modulation by corticotropin releasing factor receptors in the ventral tegmental area. Psychopharmacology (Berl). 2015;232(24):4469–79. DOI: 10.1007/s00213-015-4082-z. PMID: 26403083; PMCID: PMC4651830
87. Davis M, Hitchcock JM, Bowers MB, Berridge CW, Melia KR, Roth RH. Stress-induced activation of prefrontal cortex dopamine turnover: blockade by lesions of the amygdala. Brain Res. 1994;664(1-2):207–10. DOI: 10.1016/0006-8993(94)91972-0. PMID: 7895029
88. Wanat MJ, Hopf FW, Stuber GD, Phillips PE, Bonci A. Corticotropin-releasing factor increases mouse ventral tegmental area dopamine neuron firing through a protein kinase C-dependent enhancement of Ih. J Physiol. 2008;586(8):2157–70. DOI: 10.1113/jphysiol.2007.150078. PMID: 18308824; PMCID: PMC2465205
89. Mor D, Becchi S, Bowring J, Tsoukalas M, Balleine BW. CRF-receptor1 modulation of the dopamine projection to prelimbic cortex facilitates cognitive flexibility after acute and chronic stress. Neurobiol Stress. 2022;16:100424. DOI: 10.1016/j.ynstr.2021.100424. PMID: 35005102; PMCID: PMC8718497
90. Mantsch JR. Corticotropin releasing factor and drug seeking in substance use disorders: preclinical evidence and translational limitations. Addict Neurosci. 2022;4:100038. DOI: 10.1016/j.addicn.2022.100038. PMID: 36531188; PMCID: PMC9757758
91. Tidey JW, Miczek KA. Social defeat stress selectively alters mesocorticolimbic dopamine release: an in vivo microdialysis study. Brain Res. 1996;721(1-2):140–9. DOI: 10.1016/0006-8993(96)00159-x. PMID: 8793094
92. Phillips AG, Barr AM. Effects of chronic mild stress on motivation for sucrose: mixed messages. Psychopharmacology (Berl). 1997;134(4):361–6; discussion 71-7. DOI: 10.1007/s002130050469. PMID: 9452176
93. Hollon NG, Burgeno LM, Phillips PE. Stress effects on the neural substrates of motivated behavior. Nat Neurosci. 2015;18(10):1405–12. DOI: 10.1038/nn.4114. PMID: 26404715. PMCID: PMC4721524
94. Douma EH, de Kloet ER. Stress-induced plasticity and functioning of ventral tegmental dopamine neurons. Neurosci Biobehav Rev. 2020;108:48–77. DOI: 10.1016/j.neubiorev.2019.10.015. PMID: 31666179
95. Korotkova TM, Brown RE, Sergeeva OA, Ponomarenko AA, Haas HL. Effects of arousal- and feeding-related neuropeptides on dopaminergic and GABAergic neurons in the ventral tegmental area of the rat. Eur J Neurosci. 2006;23(10):2677–85. DOI: 10.1111/j.1460-9568.2006.04792.x. PMID: 16817870
96. Beckstead MJ, Gantz SC, Ford CP, Stenzel-Poore MP, Phillips PE, Mark GP, et al. CRF enhancement of GIRK channel-mediated transmission in dopamine neurons. Neuropsychopharmacology. 2009;34(8):1926–35. DOI: 10.1038/npp.2009.25. PMID: 19279570; PMCID: PMC3640552
97. Williams CL, Buchta WC, Riegel AC. CRF-R2 and the heterosynaptic regulation of VTA glutamate during reinstatement of cocaine seeking. J Neurosci. 2014;34(31):10402–14. DOI: 10.1523/JNEUROSCI.0911-13.2014. PMID: 25080599; PMCID: PMC4115144
98. Riegel AC, Williams JT. CRF facilitates calcium release from intracellular stores in midbrain dopamine neurons. Neuron. 2008;57(4):559–70. DOI: 10.1016/j.neuron.2007.12.029. PMID: 18304485; PMCID: PMC2696265
99. Hahn J, Hopf FW, Bonci A. Chronic cocaine enhances corticotropin-releasing factor-dependent potentiation of excitatory transmission in ventral tegmental area dopamine neurons. J Neurosci. 2009;29(20):6535–44. DOI: 10.1523/JNEUROSCI.4773-08.2009. PMID: 19458224; PMCID: PMC3077990
100. Refojo D, Schweizer M, Kuehne C, Ehrenberg S, Thoeringer C, Vogl AM, et al. Glutamatergic and dopaminergic neurons mediate anxiogenic and anxiolytic effects of CRHR1. Science. 2011;333(6051):1903–7. DOI: 10.1126/science.1202107. PMID: 21885734
101. Ungless MA, Singh V, Crowder TL, Yaka R, Ron D, Bonci A. Corticotropin-releasing factor requires CRF binding protein to potentiate NMDA receptors via CRF receptor 2 in dopamine neurons. Neuron. 2003;39(3):401–7. DOI: 10.1016/s0896-6273(03)00461-6. PMID: 12895416
102. Parham KL, Zervou S, Karteris E, Catalano RD, Old RW, Hillhouse EW. Promoter analysis of human corticotropin-releasing factor (CRF) type 1 receptor and regulation by CRF and urocortin. Endocrinology. 2004;145(8):3971–83. DOI: 10.1210/en.2004-0194. PMID: 15142984
103. Zalachoras I, Astori S, Meijer M, Grosse J, Zanoletti O, de Suduiraut IG, et al. Opposite effects of stress on effortful motivation in high and low anxiety are

mediated by CRHR1 in the VTA. Sci Adv. 2022;8(12):eabj9019. DOI: 10.1126/sciadv.abj9019. PMID: 35319997; PMCID: PMC8942367
104. Bryce CA, Floresco SB. Perturbations in effort-related decision-making driven by acute stress and corticotropin-releasing factor. Neuropsychopharmacology. 2016;41(8):2147–59. DOI: 10.1038/npp.2016.15. PMID: 26830960; PMCID: PMC4908645
105. Wanat MJ, Bonci A, Phillips PE. CRF acts in the midbrain to attenuate accumbens dopamine release to rewards but not their predictors. Nat Neurosci. 2013;16(4):383–5. DOI: 10.1038/nn.3335. PMID: 23416448; PMCID: PMC3609940
106. Bouarab C, Thompson B, Polter AM. VTA GABA neurons at the interface of stress and reward. Front Neural Circuits. 2019;13:78. DOI: 10.3389/fncir.2019.00078. PMID: 31866835; PMCID: PMC6906177
107. Zhou Z, Liu X, Chen S, Zhang Z, Liu Y, Montardy Q, et al. A VTA GABAergic neural circuit mediates visually evoked innate defensive responses. Neuron. 2019;103(3):473–88.e6. DOI: 10.1016/j.neuron.2019.05.027. PMID: 31202540
108. Tan KR, Yvon C, Turiault M, Mirzabekov JJ, Doehner J, Labouebe G, et al. GABA neurons of the VTA drive conditioned place aversion. Neuron. 2012;73(6):1173–83. DOI: 10.1016/j.neuron.2012.02.015. PMID: 22445344; PMCID: PMC6690362
109. Yu X, Zhao G, Wang D, Wang S, Li R, Li A, et al. A specific circuit in the midbrain detects stress and induces restorative sleep. Science. 2022;377(6601):63–72. DOI: 10.1126/science.abn0853. PMID: 35771921; PMCID: PMC7612951
110. Eban-Rothschild A, Borniger JC, Rothschild G, Giardino WJ, Morrow JG, de Lecea L. Arousal State-dependent alterations in VTA-GABAergic neuronal activity. eNeuro. 2020;7(2):ENEURO.0356-19.2020. DOI: 10.1523/ENEURO.0356-19.2020. PMID: 32054621; PMCID: PMC7218005
111. Cohen JY, Haesler S, Vong L, Lowell BB, Uchida N. Neuron-type-specific signals for reward and punishment in the ventral tegmental area. Nature. 2012;482(7383):85–8. DOI: 10.1038/nature10754. PMID: 22258508; PMCID: PMC3271183
112. van Zessen R, Phillips JL, Budygin EA, Stuber GD. Activation of VTA GABA neurons disrupts reward consumption. Neuron. 2012;73(6):1184–94. DOI: 10.1016/j.neuron.2012.02.016. PMID: 22445345; PMCID: PMC3314244
113. Wang HL, Morales M. Corticotropin-releasing factor binding protein within the ventral tegmental area is expressed in a subset of dopaminergic neurons. J Comp Neurol. 2008;509(3):302–18. DOI: 10.1002/cne.21751. PMID: 18478589; PMCID: PMC2575090
114. Juarez B, Kong MS, Jo YS, Elum JE, Yee JX, Ng-Evans S, et al. Temporal scaling of dopamine neuron firing and dopamine release by distinct ion channels shape behavior. Sci Adv. 2023;9(32):eadg8869. DOI: 10.1126/sciadv.adg8869. PMID: 37566654; PMCID: PMC10421029
115. Wolfart J, Neuhoff H, Franz O, Roeper J. Differential expression of the small-conductance, calcium-activated potassium channel SK3 is critical for pacemaker control in dopaminergic midbrain neurons. J Neurosci. 2001;21(10):3443–56. DOI: 10.1523/JNEUROSCI.21-10-03443.2001. PMID: 11331374; PMCID: PMC6762487
116. Azcorra M, Gaertner Z, Davidson C, He Q, Kim H, Nagappan S, et al. Unique functional responses differentially map onto genetic subtypes of dopamine neurons. Nat Neurosci. 2023;26(10):1762–74. DOI: 10.1038/s41593-023-01401-9. PMID: 37537242; PMCID: PMC10545540
117. Luppi MP, Azcorra M, Caronia-Brown G, Poulin JF, Gaertner Z, Gatica S, et al. Sox6 expression distinguishes dorsally and ventrally biased dopamine neurons in the substantia nigra with distinctive properties and embryonic origins. Cell Rep. 2021;37(6):109975. DOI: 10.1016/j.celrep.2021.109975. PMID: 34758317; PMCID: PMC8607753
118. Lammel S, Ion DI, Roeper J, Malenka RC. Projection-specific modulation of dopamine neuron synapses by aversive and rewarding stimuli. Neuron. 2011;70(5):855–62. DOI: 10.1016/j.neuron.2011.03.025. PMID: 21658580; PMCID: PMC3112473
119. Beier KT, Steinberg EE, DeLoach KE, Xie S, Miyamichi K, Schwarz L, et al. Circuit architecture of VTA dopamine neurons revealed by systematic input-output mapping. Cell. 2015;162(3):622–34. DOI: 10.1016/j.cell.2015.07.015. PMID: 26232228; PMCID: PMC4522312
120. Lerner TN, Shilyansky C, Davidson TJ, Evans KE, Beier KT, Zalocusky KA, et al. Intact-brain analyses reveal distinct information carried by SNc dopamine subcircuits. Cell. 2015;162(3):635–47. DOI: 10.1016/j.cell.2015.07.014. PMID: 26232229; PMCID: PMC4790813
121. Poulin JF, Caronia G, Hofer C, Cui Q, Helm B, Ramakrishnan C, et al. Mapping projections of molecularly defined dopamine neuron subtypes using intersectional genetic approaches. Nat Neurosci. 2018;21(9):1260–71. DOI: 10.1038/s41593-018-0203-4. PMID: 30104732. PMCID: PMC6342021
122. Haber SN, Fudge JL, McFarland N. Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. J Neurosci. 2000;20:2369–82. DOI: 10.1523/JNEUROSCI.20-06-02369.2000. PMID: 10704511; PMCID: PMC6772499
123. Steinkellner T, Conrad WS, Kovacs I, Rissman RA, Lee EB, Trojanowski JQ, et al. Dopamine neurons exhibit emergent glutamatergic identity in Parkinson's disease. Brain. 2022;145(3):879–86. DOI: 10.1093/brain/awab373. PMID: 35258081; PMCID: PMC9050538
124. Reyes S, Cottam V, Kirik D, Double KL, Halliday GM. Variability in neuronal expression of dopamine receptors and transporters in the substantia nigra. Mov Disord. 2013;28(10):1351–9. DOI: 10.1002/mds.25493. PMID: 23674405
125. Bjorklund A, Dunnett SB. Dopamine neuron systems in the brain: an update. Trends Neurosci. 2007;30(5):194–202. DOI: 10.1016/j.tins.2007.03.006. PMID: 17408759
126. McRitchie DA, Hardman CD, Halliday GM. Cytoarchitectural distribution of calcium binding proteins in midbrain dopaminergic regions of rats and humans. J Comp Neurol. 1996;364(1):121–50. DOI: 10.1002/(SICI)1096-9861(19960101)364:1⟨121::AID-CNE11⟩3.0.CO;2-1. PMID: 8789281
127. Reyes S, Fu Y, Double K, Thompson L, Kirik D, Paxinos G, et al. GIRK2 expression in dopamine neurons of the substantia nigra and ventral tegmental area. J Comp Neurol. 2012;520(12):2591–607. DOI: 10.1002/cne.23051. PMID: 22252428
128. Garritsen O, van Battum EY, Grossouw LM, Pasterkamp RJ. Development, wiring and function of dopamine neuron subtypes. Nat Rev Neurosci. 2023;24(3):134–52. DOI: 10.1038/s41583-022-00669-3. PMID: 36653531
129. Schultz W, Apicella P, Ljungberg T. Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. J Neurosci. 1993;13(3):900–13. DOI: 10.1523/JNEUROSCI.13-03-00900.1993. PMID: 8441015; PMCID: PMC6576600
130. Kobayashi S, Schultz W. Influence of reward delays on responses of dopamine neurons. J Neurosci. 2008;28(31):7837-46. DOI: 10.1523/JNEUROSCI.1600-08.2008. PMID: 18667616; PMCID: PMC3844811
131. Matsumoto M, Hikosaka O. Two types of dopamine neuron distinctly convey positive and negative motivational signals. Nature. 2009;459(7248):837-41. DOI: 10.1038/nature08028. PMID: 19448610; PMCID: PMC2739096
132. Matsumoto M, Takada M. Distinct representations of cognitive and motivational signals in midbrain dopamine neurons. Neuron. 2013;79(5):1011–24. DOI: 10.1016/j.neuron.2013.07.002. PMID: 23932490
133. Bromberg-Martin ES, Matsumoto M, Hikosaka O. Dopamine in motivational control: rewarding, aversive, and alerting. Neuron. 2010;68(5):815–34. DOI: 10.1016/j.neuron.2010.11.022. PMID: 21144997; PMCID: PMC3032992
134. Pignatelli M, Bonci A. Role of dopamine neurons in reward and aversion: A synaptic plasticity perspective. Neuron. 2015;86(5):1145–57. DOI: 10.1016/j.neuron.2015.04.015. PMID: 26050034
135. Volman SF, Lammel S, Margolis EB, Kim Y, Richard JM, Roitman MF, et al. New insights into the specificity and plasticity of reward and aversion encoding in the mesolimbic system. J Neurosci. 2013;33(45):17569–76. DOI: 10.1523/JNEUROSCI.3250-13.2013. PMID: 24198347; PMCID: PMC3818538
136. Verharen JPH, Zhu Y, Lammel S. Aversion hot spots in the dopamine system. Curr Opin Neurobiol. 2020;64:46–52. DOI: 10.1016/j.conb.2020.02.002. PMID: 32146296; PMCID: PMC7483159
137. Fu Y, Paxinos G, Watson C, Halliday GM. The substantia nigra and ventral tegmental dopaminergic neurons from development to degeneration. J Chem Neuroanat. 2016;76(Pt B):98–107. DOI: 10.1016/j.jchemneu.2016.02.001. PMID: 26859066
138. Cho YT, Fudge JL. Heterogeneous dopamine populations project to specific subregions of the primate amygdala. Neuroscience. 2010;165(4):1501–18. DOI: 10.1016/j.neuroscience.2009.11.004. PMID: 19914353; PMCID: PMC2814979
139. Barker DJ, Root DH, Zhang S, Morales M. Multiplexed neurochemical signaling by neurons of the ventral tegmental area. J Chem Neuroanat. 2016;73:33–42. DOI: 10.1016/j.jchemneu.2015.12.016. PMID: 26763116 PMCID: PMC4818729
140. Kelly EA, Contraras JM, Duan A, Vassell R, Fudge JL. Unbiased stereological estimates of dopaminergic and GABAergic neurons in the A10, A9, and A8 subregions in the young male Macaque. Neuroscience. 2022;496(496):152–64. DOI: 10.1016/j.neuroscience.2022.06.018. PMID: 35738547; PMCID: PMC9329254
141. Nair-Roberts RG, Chatelain-Badie SD, Benson E, White-Cooper H, Bolam JP, Ungless MA. Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. Neuroscience. 2008;152(4):1024–31. DOI: 10.1016/j.neuroscience.2008.01.046. PMID: 18355970; PMCID: PMC2575227
142. Trudeau LE, Hnasko TS, Wallen-Mackenzie A, Morales M, Rayport S, Sulzer D. The multilingual nature of dopamine neurons. Prog Brain Res. 2014;211:141–64. DOI: 10.1016/B978-0-444-63425-2.00006-4. PMID: 24968779; PMCID: PMC4565795
143. Mugnaini E, Oertel WH. An atlas of the distribution of GABAergic neurons and terminals in the rat CNS as revealed by GAD immunohistochemistry. In:

Bjorklund A, Hokfelt T, editors. Handbook of Chemical Neuroanatomy Part I, GABA and neuropeptides in the CNS. Elsevier Science Publishers BV; 1985. p. 436–608.
144. Yamaguchi T, Sheen W, Morales M. Glutamatergic neurons are present in the rat ventral tegmental area. Eur J Neurosci. 2007;25(1):106–18. DOI: 10.1111/j.1460-9568.2006.05263.x. PMID: 17241272; PMCID: PMC3209508
145. Yamaguchi T, Wang HL, Li X, Ng TH, Morales M. Mesocorticolimbic glutamatergic pathway. J Neurosci. 2011;31(23):8476–90. DOI: 10.1523/JNEUROSCI.1598-11.2011. PMID: 21653852; PMCID: PMC6623324
146. Root DH, Mejias-Aponte CA, Zhang S, Wang HL, Hoffman AF, Lupica CR, et al. Single rodent mesohabenular axons release glutamate and GABA. Nat Neurosci. 2014;17(11):1543–51. DOI: 10.1038/nn.3823. PMID: 25242304; PMCID: PMC4843828
147. Mendez JA, Bourque MJ, Dal Bo G, Bourdeau ML, Danik M, Williams S, et al. Developmental and target-dependent regulation of vesicular glutamate transporter expression by dopamine neurons. J Neurosci. 2008;28(25):6309–18. DOI: 10.1523/JNEUROSCI.1331-08.2008. PMID: 18562601; PMCID: PMC6670902
148. Hökfelt T, Rehfeld JF, Skirboll L, Ivemark B, Goldstein M, Markey K. Evidence for coexistence of dopamine and CCK in meso-limbic neurones. Nature. 1980;285(5765):476–8. DOI: 10.1038/285476a0. PMID: 6105617
149. Seroogy KB, Dangaran K, Lim S, Haycock JW, Fallon JH. Ventral mesencephalic neurons containing both cholecystokinin- and tyrosine hydroxylase-like immunoreactivities project to forebrain regions. J Comp Neurol. 1989;279(3):397–414. DOI: 10.1002/cne.902790306. PMID: 2563737
150. Skirboll LR, Grace AA, Hommer DW, Rehfeld J, Goldstein M, Hokfelt T, et al. Peptide-monoamine coexistence: studies of the actions of cholecystokinin-like peptide on the electrical activity of midbrain dopamine neurons. Neuroscience. 1981;6(11):2111–24. DOI: 10.1016/0306-4522(81)90002-6. PMID: 6120481
151. Anderegg A, Poulin JF, Awatramani R. Molecular heterogeneity of midbrain dopaminergic neurons–moving toward single cell resolution. FEBS Lett. 2015;589(24 Pt A):3714–26. DOI: 10.1016/j.febslet.2015.10.022. PMID: 26505674; PMCID: PMC4679573
152. Gomez JA, Perkins JM, Beaudoin GM, Cook NB, Quraishi SA, Szoeke EA, et al. Ventral tegmental area astrocytes orchestrate avoidance and approach behavior. Nat Commun. 2019;10(1):1455. DOI: 10.1038/s41467-019-09131-y. PMID: 30926783; PMCID: PMC6440962
153. Xin W, Edwards N, Bonci A. VTA dopamine neuron plasticity – the unusual suspects. Eur J Neurosci. 2016;44(12):2975–83. DOI: 10.1111/ejn.13425. PMID: 27711998; PMCID: PMC11466316
154. De Biase LM, Schuebel KE, Fusfeld ZH, Jair K, Hawes IA, Cimbro R, et al. Local cues establish and maintain region-specific phenotypes of Basal ganglia microglia. Neuron. 2017;95(2):341–56.e6. DOI: 10.1016/j.neuron.2017.06.020. PMID: 28689984; PMCID: PMC5754189
155. Mayhew J, Beart PM, Walker FR. Astrocyte and microglial control of glutamatergic signalling: a primer on understanding the disruptive role of chronic stress. J Neuroendocrinol. 2015;27(6):498–506. DOI: 10.1111/jne.12273. PMID: 25737228
156. Xin W, Schuebel KE, Jair KW, Cimbro R, De Biase LM, Goldman D, et al. Ventral midbrain astrocytes display unique physiological features and sensitivity to dopamine D2 receptor signaling. Neuropsychopharmacology. 2019;44(2):344–55. DOI: 10.1038/s41386-018-0151-4. PMID: 30054584; PMCID: PMC6300565
157. Oertel WH, Tappaz ML, Berod A, Mugnaini E. Two-color immunohistochemistry for dopamine and GABA neurons in rat substantia nigra and zona incerta. Brain Res Bull. 1982;9:463–74. DOI: 10.1016/0361-9230(82)90155-1. PMID: 6129046
158. Margolis EB, Toy B, Himmels P, Morales M, Fields HL. Identification of rat ventral tegmental area GABAergic neurons. PLoS One. 2012;7(7):e42365. DOI: 10.1371/journal.pone.0042365. PMID: 22860119; PMCID: PMC3409171
159. Nagai T, McGeer PL, McGeer EG. Distribution of GABA-T-intensive neurons in the rat forebrain and midbrain. J Comp Neurol. 1983;218:220–38. DOI: 10.1002/cne.902180209. PMID: 6886073
160. Olson VG, Nestler EJ. Topographical organization of GABAergic neurons within the ventral tegmental area of the rat. Synapse. 2007;61(2):87–95. DOI: 10.1002/syn.20345. PMID: 17117419
161. Yang J, Chen J, Liu Y, Chen KH, Baraban JM, Qiu Z. Ventral tegmental area astrocytes modulate cocaine reward by tonically releasing GABA. Neuron. 2023;111(7):1104–17.e6. DOI: 10.1016/j.neuron.2022.12.033. PMID: 36681074; PMCID: PMC10079641
162. Breton JM, Charbit AR, Snyder BJ, Fong PTK, Dias EV, Himmels P, et al. Relative contributions and mapping of ventral tegmental area dopamine and GABA neurons by projection target in the rat. J Comp Neurol. 2019;527(5):916–41. DOI: 10.1002/cne.24572. PMID: 30393861; PMCID: PMC6347508
163. Carr DB, Sesack SR. GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex. Synapse. 2000;38(2):114–23. DOI: 10.1002/1098-2396(200011)38:2⟨114::AID-SYN2⟩3.0.CO;2-R. PMID: 11018785
164. Omelchenko N, Sesack SR. Ultrastructural analysis of local collaterals of rat ventral tegmental area neurons: GABA phenotype and synapses onto dopamine and GABA cells. Synapse. 2009;63(10):895–906. DOI: 10.1002/syn.20668. PMID: 19582784; PMCID: PMC2741309
165. Steffensen SC, Svingos AL, Pickel VM, Henriksen SJ. Electrophysiological characterization of GABAergic neurons in the ventral tegmental area. J Neurosci. 1998;18(19):8003–15. DOI: 10.1523/JNEUROSCI.18-19-08003.1998. PMID: 9742167; PMCID: PMC6793009
166. Taylor SR, Badurek S, Dileone RJ, Nashmi R, Minichiello L, Picciotto MR. GABAergic and glutamatergic efferents of the mouse ventral tegmental area. J Comp Neurol. 2014;522(14):3308–34. DOI: 10.1002/cne.23603. PMID: 24715505; PMCID: PMC4107038
167. Van Bockstaele EJ, Pickel VM. GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. Brain Res. 1995;682(1-2):215–21. DOI: 10.1016/0006-8993(95)00334-m. PMID: 7552315
168. Parent A, Côté PY, Lavoie B. Chemical anatomy of primate basal ganglia. Prog Neurobiol. 1995;46(2–3):131–97. PMID: 7568912
169. Paul EJ, Kalk E, Tossell K, Irvine EE, Franks NP, Wisden W, et al. nNOS-expressing neurons in the ventral tegmental area and substantia nigra pars compacta. eNeuro. 2018;5(5):ENEURO.0381-18.2018. DOI: 10.1523/ENEURO.0381-18.2018. PMID: 30456293; PMCID: PMC6240015
170. Nagaeva E, Zubarev I, Bengtsson Gonzales C, Forss M, Nikouei K, de Miguel E, et al. Heterogeneous somatostatin-expressing neuron population in mouse ventral tegmental area. Elife. 2020;9:e59328. DOI: 10.7554/eLife.59328. PMID: 32749220; PMCID: PMC7440918
171. Morales M, Margolis EB. Ventral tegmental area: cellular heterogeneity, connectivity and behaviour. Nat Rev Neurosci. 2017;18(2):73–85. DOI: 10.1038/nrn.2016.165. PMID: 28053327
172. González-Hernández T, Barroso-Chinea P, Acevedo A, Salido E, Rodríguez M. Colocalization of tyrosine hydroxylase and GAD65 mRNA in mesostriatal neurons. Eur J Neurosci. 2001;13(1):57–67. PMID: 11135004
173. Kim JI, Ganesan S, Luo SX, Wu YW, Park E, Huang EJ, et al. Aldehyde dehydrogenase 1a1 mediates a GABA synthesis pathway in midbrain dopaminergic neurons. Science. 2015;350(6256):102–6. DOI: 10.1126/science.aac4690. PMID: 26430123; PMCID: PMC4725325
174. Tritsch NX, Oh WJ, Gu C, Sabatini BL. Midbrain dopamine neurons sustain inhibitory transmission using plasma membrane uptake of GABA, not synthesis. Elife. 2014;3:e01936. DOI: 10.7554/eLife.01936. PMID: 24843012; PMCID: PMC4001323
175. Sulzer D, Joyce MP, Lin L, Geldwert D, Haber SN, Hattori T, et al. Dopamine neurons make Glutamatergic synapses in vitro. J Neurosci. 1998;18(12):4588–602. DOI: 10.1523/JNEUROSCI.18-12-04588.1998. PMID: 9614234; PMCID: PMC6792695
176. Dal Bo G, Berube-Carriere N, Mendez JA, Leo D, Riad M, Descarries L, et al. Enhanced glutamatergic phenotype of mesencephalic dopamine neurons after neonatal 6-hydroxydopamine lesion. Neuroscience. 2008;156(1):59–70. DOI: 10.1016/j.neuroscience.2008.07.032. PMID: 18706980
177. Kawano M, Kawasaki A, Sakata-Haga H, Fukui Y, Kawano H, Nogami H, et al. Particular subpopulations of midbrain and hypothalamic dopamine neurons express vesicular glutamate transporter 2 in the rat brain. J Comp Neurol. 2006;498(5):581–92. DOI: 10.1002/cne.21054. PMID: 16917821
178. Yamaguchi T, Wang HL, Morales M. Glutamate neurons in the substantia nigra compacta and retrorubral field. Eur J Neurosci. 2013;38(11):3602–10. DOI: 10.1111/ejn.12359. PMID: 24102658; PMCID: PMC3903463
179. Mingote S, Chuhma N, Kusnoor SV, Field B, Deutch AY, Rayport S. Functional connectome analysis of dopamine neuron glutamatergic connections in forebrain regions. J neurosci. 2015;35(49):16259–71. DOI: 10.1523/JNEUROSCI.1674-15.2015. PMID: 26658874; PMCID: PMC4682788
180. Boulland JL, Qureshi T, Seal RP, Rafiki A, Gundersen V, Bergersen LH, et al. Expression of the vesicular glutamate transporters during development indicates the widespread corelease of multiple neurotransmitters. J Comp Neurol. 2004;480(3):264–80. DOI: 10.1002/cne.20354. PMID: 15515175
181. Bayer SA, Wills KV, Triarhou LC, Ghetti B. Time of neuron origin and gradients of neurogenesis in midbrain dopaminergic neurons in the mouse. Exp Brain Res. 1995;105(2):191–9. DOI: 10.1007/BF00240955. PMID: 7498372
182. Brignani S, Pasterkamp RJ. Neuronal subset-specific migration and axonal wiring mechanisms in the developing midbrain dopamine system. Front Neuroanat. 2017;11:55. DOI: 10.3389/fnana.2017.00055. PMID: 28740464; PMCID: PMC5502286
183. Steinkellner T, Zell V, Farino ZJ, Sonders MS, Villeneuve M, Freyberg RJ, et al. Role for VGLUT2 in selective vulnerability of midbrain dopamine neurons. J Clin

Invest. 2018;128(2):774–88. DOI: 10.1172/JCI95795. PMID: 29337309; PMCID: PMC5785252
184. Bérubé-Carrière N, Riad M, Dal Bo G, Lévesque D, Trudeau LE, Descarries L. The dual dopamine-glutamate phenotype of growing mesencephalic neurons regresses in mature rat brain. J Comp Neurol. 2009;517(6):873–91. DOI: 10.1002/cne.22194. PMID: 19844994
185. Root DH, Wang HL, Liu B, Barker DJ, Mód L, Szocsics P, et al. Glutamate neurons are intermixed with midbrain dopamine neurons in nonhuman primates and humans. Sci Rep. 2016;6:30615. DOI: 10.1038/srep30615. PMID: 27477243; PMCID: PMC4967922
186. Yamaguchi T, Qi J, Wang HL, Zhang S, Morales M. Glutamatergic and dopaminergic neurons in the mouse ventral tegmental area. Eur J Neurosci. 2015;41(6):760–72. DOI: 10.1111/ejn.12818. PMID: 25572002; PMCID: PMC4363208
187. Conrad WS, Oriol L, Kollman GJ, Faget L, Hnasko TS. Proportion and distribution of neurotransmitter-defined cell types in the ventral tegmental area and substantia nigra pars compacta. bioRxiv. 2024. DOI: 10.1101/2024.02.28.582356. PMID: 38464250; PMCID: PMC10925288
188. Poulin JF, Zou J, Drouin-Ouellet J, Kim KY, Cicchetti F, Awatramani RB. Defining midbrain dopaminergic neuron diversity by single-cell gene expression profiling. Cell Rep. 2014;9(3):930–43. DOI: 10.1016/j.celrep.2014.10.008. PMID: 25437550; PMCID: PMC4251558
189. La Manno G, Gyllborg D, Codeluppi S, Nishimura K, Salto C, Zeisel A, et al. Molecular diversity of midbrain development in mouse, Human, and stem cells. Cell. 2016;167(2):566–80.e19. DOI: 10.1016/j.cell.2016.09.027. PMID: 27716510; PMCID: PMC5055122
190. Tiklová K, Björklund ÅK, Lahti L, Fiorenzano A, Nolbrant S, Gillberg L, et al. Single-cell RNA sequencing reveals midbrain dopamine neuron diversity emerging during mouse brain development. Nat Commun. 2019;10(1):581. DOI: 10.1038/s41467-019-08453-1. PMID: 30718509; PMCID: PMC6362095
191. Pan P-Y, Ryan TA. Calbindin controls release probability in ventral tegmental area dopamine neurons. Nat Neurosci. 2012;15:813–5. DOI: 10.1038/nn.3099. PMID: 22544312; PMCID: PMC3703651
192. Evans RC. Dendritic involvement in inhibition and disinhibition of vulnerable dopaminergic neurons in healthy and pathological conditions. Neurobiol Dis. 2022;172:105815. DOI: 10.1016/j.nbd.2022.105815. PMID: 35820645; PMCID: PMC9851599
193. Haber SN, Ryoo H, Cox C, Lu W. Subsets of midbrain dopaminergic neurons in monkeys are distinguished by different levels of mRNA for the dopamine transporter: comparison with the mRNA for the D2 receptor, tyrosine hydroxylase and calbindin immunoreactivity. J Comp Neurol. 1995;362(3):400–10. DOI: 10.1002/cne.903620308. PMID: 8576447
194. Poulin JF, Gaertner Z, Moreno-Ramos OA, Awatramani R. Classification of midbrain dopamine neurons using single-cell gene expression profiling approaches. Trends Neurosci. 2020;43(3):155–69. DOI: 10.1016/j.tins.2020.01.004. PMID: 32101709; PMCID: PMC7285906
195. Williams SM, Goldman-Rakic PS. Widespread origin of the primate mesofrontal dopamine system. Cereb Cortex. 1998;8(4):321–45. DOI: 10.1093/cercor/8.4.321. PMID: 9651129
196. Gaspar P, Heizmann CW, Kaas JH. Calbindin D-28K in the dopaminergic mesocortical projection of a monkey (Aotus trivirgatus). Brain Res. 1993;603(1):166–72. DOI: 10.1016/0006-8993(93)91317-l. PMID: 8095839
197. Gaspar P, Stepneiwska I, Kaas JH. Topography and collateralization of the dopaminergic projections to motor and lateral prefrontal cortex in owl monkeys. J Comp Neurol. 1992;325:1–21. DOI: 10.1002/cne.903250102. PMID: 1362430
198. Plant TM, Terasawa Ei, Witchel SF. Puberty in non-human primates and man. Physiological Control Systems and Governing Gonadal Function. 4th Edition; 2015, 1487–536.
199. Morgan DG, Finch CE. Dopaminergic changes in the basal ganglia. A generalized phenomenon of aging in mammals. Ann N Y Acad Sci. 1988;515:145–60. DOI: 10.1111/j.1749-6632.1988.tb32978.x. PMID: 3364883
200. Fortin GM, Bourque MJ, Mendez JA, Leo D, Nordenankar K, Birgner C, et al. Glutamate corelease promotes growth and survival of midbrain dopamine neurons. J Neurosci. 2012;32(48):17477–91. DOI: 10.1523/JNEUROSCI.1939-12.2012. PMID: 23197738; PMCID: PMC6621856
201. Fremeau RT Jr., Troyer MD, Pahner I, Nygaard GO, Tran CH, Reimer RJ, et al. The expression of vesicular glutamate transporters defines two classes of excitatory synapse. Neuron. 2001;31(2):247–60. DOI: 10.1016/s0896-6273(01)00344-0. PMID: 11502256
202. Hnasko TS, Chuhma N, Zhang H, Goh GY, Sulzer D, Palmiter RD, et al. Vesicular glutamate transport promotes dopamine storage and glutamate corelease in vivo. Neuron. 2010;65(5):643–56. DOI: 10.1016/j.neuron.2010.02.012. PMID: 20223200; PMCID: PMC2846457
203. Justice NJ, Yuan ZF, Sawchenko PE, Vale W. Type 1 corticotropin-releasing factor receptor expression reported in BAC transgenic mice: implications for reconciling ligand-receptor mismatch in the central corticotropin-releasing factor system. J Comp Neurol. 2008;511(4):479–96. DOI: 10.1002/cne.21848. PMID: 18853426; PMCID: PMC2597626
204. Wise RA, Morales M. A ventral tegmental CRF-glutamate-dopamine interaction in addiction. Brain Res. 2010;1314:38–43. DOI: 10.1016/j.brainres.2009.09.101. PMID: 19800323; PMCID: PMC2819620
205. Heymann G, Jo YS, Reichard KL, McFarland N, Chavkin C, Palmiter RD, et al. Synergy of distinct dopamine projection populations in behavioral reinforcement. Neuron. 2020;105(5):909–20.e5. DOI: 10.1016/j.neuron.2019.11.024. PMID: 31879163; PMCID: PMC7060117
206. Sharpe AL, Trzeciak M, Eliason NL, Blankenship HE, Byrd BAM, Douglas PD, et al. Repeated cocaine or methamphetamine treatment alters astrocytic CRF2 and GLAST expression in the ventral midbrain. Addict Biol. 2022;27(2):e13120. DOI: 10.1111/adb.13120. PMID: 34825430; PMCID: PMC9872560
207. Zoli M, Torri C, Ferrari R, Jansson A, Zini I, Fuxe K, et al. The emergence of the volume transmission concept. Brain Res Brain Res Rev. 1998;26(2-3):136–47. DOI: 10.1016/s0165-0173(97)00048-9. PMID: 9651506
208. Jiang Z, Rajamanickam S, Justice NJ. Local corticotropin-releasing factor signaling in the hypothalamic paraventricular nucleus. J Neurosci. 2018;38(8):1874–90. DOI: 10.1523/JNEUROSCI.1492-17.2017. PMID: 29352046; PMCID: PMC5824736
209. Hökfelt T. Neuropeptides in perspective: the last ten years. Neuron. 1991;7(6):867–79. DOI: 10.1016/0896-6273(91)90333-u. PMID: 1684901
210. van den Pol AN. Neuropeptide transmission in brain circuits. Neuron. 2012;76(1):98–115. DOI: 10.1016/j.neuron.2012.09.014. PMID: 23040809; PMCID: PMC3918222
211. Ferré S. Hormones and neuropeptide receptor heteromers in the ventral tegmental area. Targets for the treatment of loss of control of food intake and substance use disorders. Curr Treat Options Psychiatry. 2017;4(2):167–83. DOI: 10.1007/s40501-017-0109-x. PMID: 28580231; PMCID: PMC5432584
212. Chen Y, Kramár EA, Chen LY, Babayan AH, Andres AL, Gall CM, et al. Impairment of synaptic plasticity by the stress mediator CRH involves selective destruction of thin dendritic spines via RhoA signaling. Mol Psychiatry. 2013;18(4):485–96. DOI: 10.1038/mp.2012.17. PMID: 22411227; PMCID: PMC3440527
213. Gounko NV, Swinny JD, Kalicharan D, Jafari S, Corteen N, Seifi M, et al. Corticotropin-releasing factor and urocortin regulate spine and synapse formation: structural basis for stress-induced neuronal remodeling and pathology. Mol Psychiatry. 2013;18(1):86–92. DOI: 10.1038/mp.2012.43. PMID: 22547117
214. Vandael D, Wierda K, Vints K, Baatsen P, De Groef L, Moons L, et al. Corticotropin-releasing factor induces functional and structural synaptic remodelling in acute stress. Transl Psychiatry. 2021;11(1):378. DOI: 10.1038/s41398-021-01497-2. PMID: 34234103; PMCID: PMC8263770
215. Curran MM, Sandman CA, Poggi Davis E, Glynn LM, Baram TZ. Abnormal dendritic maturation of developing cortical neurons exposed to corticotropin releasing hormone (CRH): insights into effects of prenatal adversity? PLoS One. 2017;12(6):e0180311. DOI: 10.1371/journal.pone.0180311. PMID: 28658297; PMCID: PMC5489219

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RESEARCH ARTICLE

Deciphering the molecular basis of accelerated biological aging in substance use disorder: Integrative transcriptomic analysis

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Substance use disorders (SUDs) contribute to early-onset age-related diseases and represent a major global health burden. Accelerated biological aging (AA) has been proposed as a key factor behind SUD-related morbidity and mortality. This study aimed to elucidate the molecular basis of AA in SUD by analyzing transcriptomic profiles in postmortem dorsolateral prefrontal cortex tissue from individuals with SUD, including alcohol (AUD), opioid (OUD), and stimulant use disorders (StUD). We examined brain tissue from 58 donors to assess differential aging patterns and AA across SUD using epigenetic clocks specifically designed for brain tissues (DNAmClock$_{Cortical}$, CerebralCortexClock$_{common}$, and PCBrainAge). Samples were then stratified into those with and without AA to perform differential expression analyses across groups and to identify biological pathways potentially related to AA. Analyses identified multiple differentially expressed genes linked to AA, revealing unique and overlapping biological pathways within SUD subtypes. Further, our analysis highlighted shared aging mechanisms across SUD subtypes, particularly mitochondrial signaling and metabolic processes. While insightful, these subtype-specific findings remain exploratory due to limited statistical power. Most biological pathways underlying AA in SUD appear to be subtype-specific, with distinct molecular signatures influenced by substance type. Given the cross-sectional design, causal interpretations are limited. Further research may support targeted interventions for aging-related risks in SUD populations.

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Introduction

Substance use disorders (SUDs) lead to the early onset of age-related diseases and pose a critical global health challenge, ranking as the fifth cause of years lived with disability, the ninth cause of disability-adjusted life years, and the 15th cause of years of life lost due to premature mortality (1). SUDs are also associated with increased risks for chronic physical health conditions such as cardiovascular disease, cancer, chronic pain, and increased risk for long-term cognitive impairments (2, 3).

Biological aging is a process that describes the progressive deterioration of biological functions, in contrast to chronological aging, which represents the time since birth (4). Epigenetic clocks, such as Hannum, Horvath, PhenoAge, and GrimAge, which incorporate DNA methylation (DNAm) data from unique CpG sites across the genome into weighted linear equations to predict age and other health outcomes, are currently considered the most promising biomarkers of biological aging (5). Estimates of epigenetic accelerated aging (AA) are obtained by regressing the predicted epigenetic age against chronological age within a cohort, where positive values indicate faster-than-expected biological aging (6).

Emerging research has underscored the biological mechanisms underlying early-onset morbidity and premature mortality in SUD, with AA proposed as a potential driver of these adverse outcomes (7). This has been most consistently shown in alcohol use disorder (AUD), where patients exhibit biological ages that exceed their chronological ages and appear biologically older than controls in both brain and blood tissues when measured by the PhenoAge and Horvath clocks (8–12), and biological aging can be partly reversed with abstinence (10). Additionally, chronic heroin use has been associated with shorter DNAm-based telomere length (13). However, findings across SUDs such as stimulant use disorder (StUD) and opioid use disorder (OUD) are inconsistent. Assessments using first-generation epigenetic clocks such as Horvath and Hannum have shown no clear significant differences between these SUDs and control groups, and even counterintuitive negative biological aging has been reported (9, 12).

The absence of consistently higher AA in some SUDs does not negate its relevance; rather, the findings from current studies imply that adverse aging outcomes in SUD may be driven by distinct biological processes and that the degree of AA may vary based on substance-specific effects and the type of epigenetic clock used for assessment. In regards to the latter, although previous studies, including our own, have shown that epigenetic clocks designed for use in peripheral blood may serve as good estimators of brain aging (8), the extent of AA in SUD in the brain has not previously been comprehensively explored using epigenetic clocks specifically designed for brain tissues. In this study, we aimed to identify substance-specific transcriptomic profiles of AA in the dorsolateral prefrontal cortex (DLPFC, Brodmann area [BA] 9), a key region involved in cognitive processes relevant to SUD, such as executive functions, decision-making, behavioral and cognitive inhibition, working memory, and craving (14). Specifically, we hypothesized that distinct drug-specific biological pathways would influence AA in SUD, potentially explaining the variability in aging outcomes observed in these disorders.

In this study, we explored the relationship between SUD and epigenetic markers of AA, focusing on AUD, OUD, and StUD. By concentrating on specific SUDs, we aimed to clarify substance-specific aging patterns and minimize confounding effects that could arise from broader case–control comparisons. Our objectives were: (i) to identify differentially expressed genes (DEGs) associated with AA in individuals with SUD and (ii) to explore overlaps in enriched biological pathways and mechanisms across different SUD subtypes (AUD, OUD, and StUD) related to AA.

Results

Participant demographic, clinical, and biological characteristics are summarized in Table 1 and Supplementary Table S2. The identified AA− and

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Table 1. Sample characteristics and group comparisons

	Overall	AA−	AA+	Statistics	*p*-value	Effect size
Sample size, freq.	58	30	28			
Age, mean (SD)	45.84(14.49)	44.33(16.54)	47.46(12.01)	t[52.89] = −0.83	0.411	0.113
Sex, freq. (%)	15(25.9)	6(20.0)	9(32.1)	ï‡Â²[1] = 0.57	0.45	0.139
Smoking index, mean (SD)	0.82(0.05)	0.83(0.06)	0.81(0.05)	t[56] = 1	0.32	0.133
Race: White, freq. (%)	35(60.3)	16(53.3)	19(67.9)	ï‡Â²[1] = 0.74	0.389	0.148
Race: Black, freq. (%)	17(29.3)	9(30.0)	8(28.6)	ï‡Â²[1] = 0	0.999	0.016
Race: Hispanic, freq. (%)	6(10.3)	5(16.7)	1(3.6)	ï‡Â²[1] = 1.45	0.228	0.215
AUD, freq. (%)	13(22.4)	6(20.0)	7(25.0)	ï‡Â²[1] = 0.02	0.888	0.06
OUD, freq. (%)	16(27.6)	10(33.3)	6(21.4)	ï‡Â²[1] = 0.52	0.472	0.133
StUD, freq. (%)	10(17.2)	4(13.3)	6(21.4)	ï‡Â²[1] = 0.22	0.64	0.107
AUD and OUD, freq. (%)	6(10.3)	4(13.3)	2(7.1)	ï‡Â²[1] = 0.12	0.732	0.102
AUD and StUD, freq. (%)	5(8.6)	3(10.0)	2(7.1)	ï‡Â²[1] = 0	0.999	0.051
OUD and StUD, freq. (%)	3(5.2)	1(3.3)	2(7.1)	ï‡Â²[1] = 0	0.951	0.086
Polysubstance use disorder, freq. (%)	5(8.6)	2(6.7)	3(10.7)	ï‡Â²[1] = 0.01	0.936	0.072
Cause of death: Cardiovascular/Chronic conditions, freq. (%)	22(37.9)	11(36.7)	11(39.3)	ï‡Â²[1] = 0	0.999	0.027
Cause of death: Overdose, freq. (%)	34(58.6)	19(63.3)	15(53.6)	ï‡Â²[1] = 0.24	0.626	0.099
Cause of death: Other, freq. (%)	2(3.4)	–	2(7.1)	ï‡Â²[1] = 0.59	0.441	0.196
PCBrainAgeClockAcc, mean (SD)	0.05(3.95)	−1.87(3.79)	2.11(3.00)	t[54.59] = −4.44	0.999	0.515
DNACorticalClockAcc, mean (SD)	−0.30(3.44)	−2.42(2.65)	1.97(2.64)	t[55.74] = −6.32	0.999	0.646
CerebralCortexClockCommonAcc, mean (SD)	−0.49(3.34)	−2.65(2.18)	1.84(2.76)	t[51.38] = −6.83	0.999	0.69
PCHorvath1Acc, mean (SD)	0.00(3.79)	−0.92(4.03)	0.98(3.30)	t[55.12] = −1.97	0.054	0.256
PCHorvath2Acc, mean (SD)	0.00(2.47)	−0.62(2.60)	0.66(2.18)	t[55.37] = −2.03	0.047	0.263
PCHannumAcc, mean (SD)	0.00(1.47)	−0.17(1.53)	0.19(1.41)	t[56] = −0.94	0.353	0.124
PCPhenoAgeAcc, mean (SD)	0.00(1.73)	−0.18(1.83)	0.20(1.63)	t[55.89] = −0.84	0.405	0.112
PCGrimAgeAcc, mean (SD)	0.00(1.12)	0.12(1.04)	−0.13(1.21)	t[53.57] = 0.84	0.403	0.114
Batch, freq. (%)	20(34.5)	9(30.0)	11(39.3)	ï‡Â²[1] = 0.22	0.64	0.098
PMIhrs, freq. (%)	26.23(7.64)	27.66(8.20)	24.69(6.80)	t[55.25] = 1.51	0.138	0.199
RIN Novogene, freq. (%)	7.18(0.96)	7.03(0.99)	7.35(0.91)	t[55.98] = −1.28	0.206	0.169
pH, freq. (%)	6.54(0.28)	6.55(0.29)	6.53(0.28)	t[55.94] = 0.21	0.831	0.029
dtangle: Astrocytes, freq. (%)	0.32(0.15)	0.34(0.15)	0.30(0.16)	t[55.04] = 1.02	0.314	0.136
dtangle: Endothelia, freq. (%)	0.09(0.02)	0.08(0.02)	0.09(0.02)	t[53.01] = −0.9	0.372	0.123
dtangle: Microglia, freq. (%)	0.05(0.02)	0.05(0.01)	0.05(0.02)	t[46.69] = −0.42	0.676	0.062
dtangle: Neurons, freq. (%)	0.40(0.13)	0.39(0.12)	0.42(0.13)	t[54.76] = −0.96	0.341	0.129
dtangle: Oligodendrocytes, freq. (%)	0.07(0.03)	0.06(0.03)	0.07(0.04)	t[48.75] = −1.06	0.292	0.151
dtangle: OPCs, freq. (%)	0.07(0.02)	0.07(0.01)	0.07(0.02)	t[51.77] = 1.27	0.211	0.173

The Student *t* test was used to compare the ages of the different groups, with the effect size reported as an *r* statistic. For *r*, values of 0.1, 0.3, and 0.5 denote small, moderate, and large effects, respectively. The chi-square test was employed for categorical variables, with effect sizes reported using Cramér's V. For Cramér's V, values of 0.1, 0.3, and 0.5 indicate small, moderate, and large effects, respectively.

AA+ groups were comparable across variables such as chronological age, sex distribution, and racial composition. Supplementary Figure S2 shows the overlap of AA between SUD types.

Differential Expression and Pathway Enrichment Analyses

DEG analyses between the AA+ and AA− groups were carried out within all SUD (AA+, $n = 28$; AA−, $n = 30$) and then individually within each SUD subtype: AUD (AA+, $n = 7$; AA−, $n = 6$), OUD (AA+, $n = 6$; AA−, $n = 10$), and StUD (AA+, $n = 6$; AA−, $n = 4$). We identified 11 DEGs in the combined SUD analysis (Supplementary Table S3). At the same time, exploratory analyses in the SUD subgroups revealed 463 DEGs in primary AUD (Figure 1A; Supplementary Table S4), 58 in primary OUD (Figure 1B; Supplementary Table S5), and 51 in StUD (Figure 1C; Supplementary Table S6). Notably, only a few DEGs were shared across all SUD subgroups (Figure 1D). Gene Ontology (GO) biological process (BP) pathway analyses revealed significant enrichment in 85 pathways in AUD (Supplementary Table S7), 9 in OUD (Supplementary Table S8), and 17 in StUD (Supplementary Table S9). The top 10 pathways from the GO BP enrichment analyses conducted for all SUD subgroups in DEG are shown in Table 2. Though insightful, these subtype-specific findings remain exploratory due to limited statistical power.

SUD Subgroups Overlaps

DEG Overlaps Between AUD and OUD. Among SUD subgroups, a larger overlap in the DEGs between AA+ when compared with AA− was found for the AUD and the OUD groups (Table 3). Remarkably, most of the genes were downregulated in AA+ when compared with AA− within OUD but upregulated within AUD (*TRIOBP, TNS2, NIBAN2,* and *SOX17*), while the others had the exact opposite pattern, being upregulated in AA+ when compared with AA− within the OUD but downregulated within AUD (*RAB3C, PGM2L1,* and *ROBO2*).

DEG Overlaps Between AUD and StUD. Five DEGs were found to overlap among the AUD and StUD groups: *EDN1, HBA2, HBA1, AQP1*, and *HBB* (Table 3). Within the AUD group, all these genes were upregulated in AA+ when compared with AA−. However, within the StUD group, only *AQP1* was upregulated, while *EDN1, HBA2, HBA1*, and *HBB* were downregulated. These contrasting patterns suggest different biological responses in these groups concerning aging in AUD and StUD.

DEG Overlaps Between OUD and StUD. Two DEGs were identified as overlapping among the OUD and StUD groups (Table 3): *TTYH2* and *TMEM63A*.

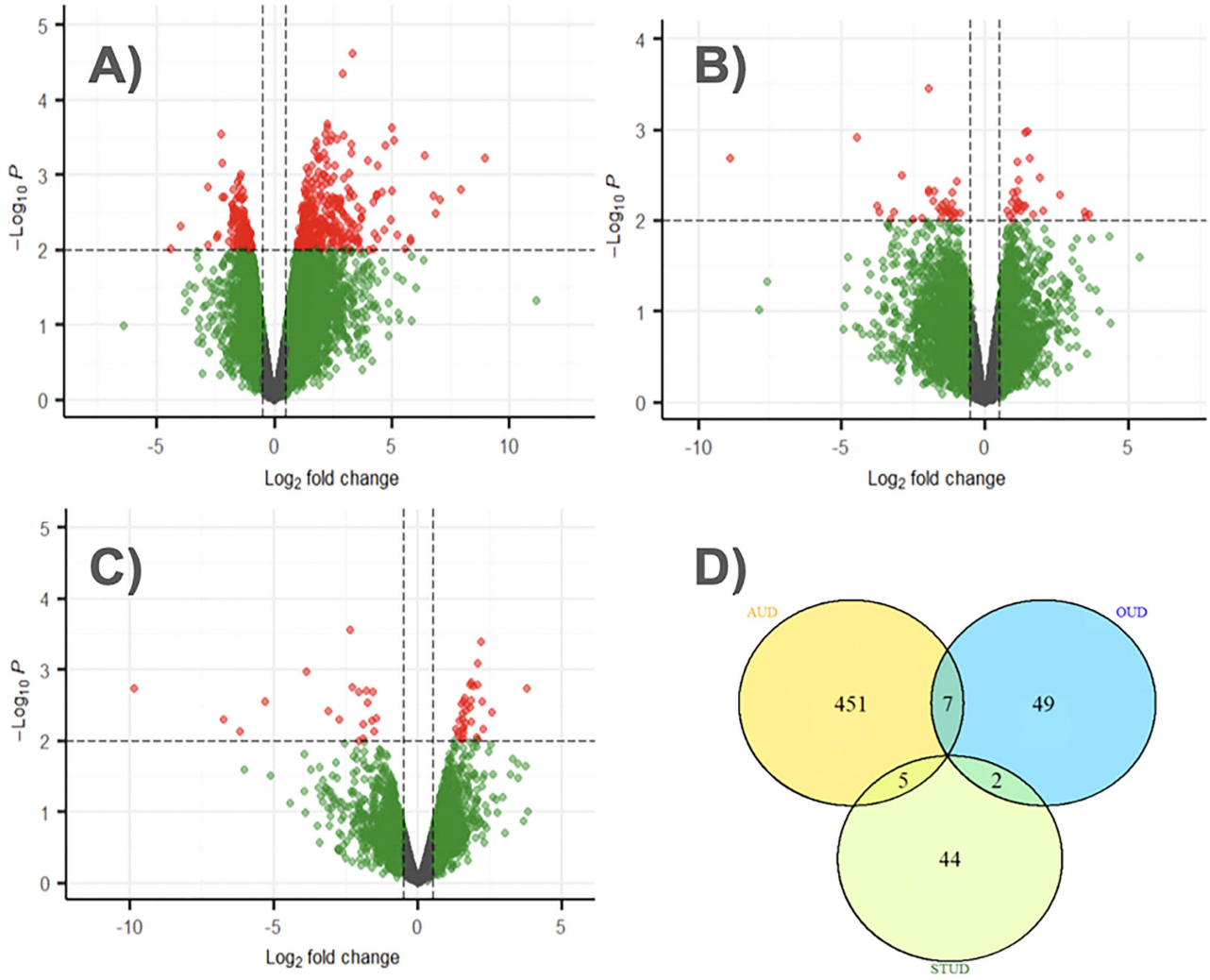

Figure 1. Differential expression in DLPFC of SUD subjects with accelerated biological aging compared to those without. Volcano plots show differences between accelerated aging positive (AA+) and accelerated aging negative (AA−) in (A) AUD(AA+, $n = 7$; AA−, $n = 6$); (B) OUD (AA+, $n = 6$; AA−, $n = 10$); and (C) StUD (AA+, $n = 6$; AA−, $n = 4$). The Venn Diagram (D) shows the overlaps between the differentially expressed genes identified. As we can see, five genes were differentially expressed when comparing AA+ and AA− in both AUD and StUD; seven genes were differentially expressed when comparing AA+ and AA− in both AUD and OUD; and two genes were differentially expressed when comparing AA+ and AA− in both OUD and StUD.

Both genes were downregulated in AA+ when compared with AA− in OUD, while in the StUD group, they were upregulated in AA+.

Molecular Mechanisms Linking Differential Gene Expression Across SUDs

Figure 2 illustrates a hypothetical molecular framework integrating DEGs from enriched pathways identified in AUD, StUD, and OUD (Table 2), highlighting key biological pathways involved in neuroinflammation, mitochondrial dysfunction, and oxidative stress as potential mechanisms underlying AA in SUD. The diagram highlights interactions among transcription factors, inflammatory mediators, and mitochondrial regulators, suggesting distinct but converging pathways contributing to cellular stress, mitochondria function, and neuroinflammation across SUD subtypes.

Discussion

To our knowledge, this is the first study investigating brain AA in SUDs using epigenetic clocks specifically designed for brain tissues ($DNAmClock_{Cortical}$, $CerebralCortexClock_{common}$, and PCBrainAge). Notably, we found that despite the high correlation between the clocks, the contribution of the variables observed in our principal component analyses (PCA), along with the innovative dichotomous classification of our sample, emphasized that the three brain-specific epigenetic clocks have distinct characteristics and do not necessarily converge when classifying individuals based on their AA. Hence, our findings align with the idea that each clock might capture unique aspects of aging. As we noted in the Method section, while this PC1-based dichotomization facilitates downstream comparisons, it represents a simplification of what is likely a continuous biological process. This classification should, therefore, be interpreted as a pragmatic, exploratory strategy to investigate broad molecular differences associated with higher versus lower levels of epigenetic aging in the brain.

Overall, the differential gene expression and pathway analysis findings suggest that AA in SUD is not a uniform process but that distinct biological mechanisms contribute to aging, depending on the type of substance involved. The most robust differences between AA+ and AA− were observed in the AUD group, which aligns with previous research showing an effect of AUD on AA (9, 12). Enrichment analyses suggest that AA is related to protein phosphorylation, signal transduction, and the positive regulation of protein localization to the plasma membrane. Protein phosphorylation and signal transduction are essential processes often altered in both normal aging and disease progression (15, 16). Furthermore, the finding of enrichment of glutamatergic synapse pathways aligns with studies suggesting a critical role of glutamate in both aging and

Table 2. Top 10 GO BP pathways identified when comparing AA+ and AA− in SUD groups

Term	Genes	Fold enrichment
Alcohol use disorder		
Positive regulation of integrin-mediated signaling pathway	LAMB2, EMP2, LIMS2	9.699*
Cellular response to zinc ion	MT2A, MT1M, MT1X, MT3, MT1E	9.429**
Intracellular zinc ion homeostasis	MT2A, SLC30A9, MT1M, MT1X, MT3, SLC39A14, MT1E	9.052***
Negative regulation of endocytosis	LGALS3, RUBCN, SYT11	9.052*
Positive regulation of G protein-coupled receptor signaling pathway	GPER1, TMOD2, SLC39A14	9.052*
Removal of superoxide radicals	NOS3, MT3, SOD3	8.487*
Regulation of store-operated calcium entry	CRACR2B, HOMER1, SLC8B1	8.487*
Positive regulation of leukocyte migration	MADCAM1, ZP3, VEGFA	8.487*
Negative regulation of viral genome replication	IFITM3, SRPK2, IFITM2, RSAD2, MX1, EIF2AK2, IFIT1	6.888***
Platelet-derived growth factor receptor signaling pathway	NR4A3, TXNIP, PTPRJ, CSPG4, PLAT	6.858**
Opioid use disorder		
Central nervous system development	ROBO2, CITED2, ZIC3, ID3	9.997**
Outer ear morphogenesis	EYA1, ZIC3	78.31***
Metanephros development	ROBO2, SOX17, EYA1, ID3	46.986***
Positive regulation of execution phase of apoptosis	TP53BP2, HTR2A	46.986***
Positive regulation of gene expression	IL32, SOX17, CSF1, CITED2, HDAC1, ID3	4.154*
Left/right axis specification	CITED2, ZIC3	3.915*
Positive regulation of DNA-templated transcription	NIBAN2, SOX17, CITED2, HDAC1, ZIC3, TRIM21, NPAS3	3.46*
Outflow tract morphogenesis	SOX17, EYA1, CITED2	19.947*
Heart looping	SOX17, CITED2, ZIC3	14.89*
Stimulant use disorder		
Central nervous system development	UGTB, RELN, MOG	8.933*
Oxygen transport	HBB, HBA2, HBA1	78.726***
Positive regulation of fibroblast migration	THBS1, AQP1	64.596**
Semaphorin-plexin signaling pathway involved in axon guidance	EDN1, PLXNB3	55.983*
Cell adhesion	CLDN11, MAG, RELN, MOG, PCDHGB2, CCN1, THBS1	5.685*
Transport	ALB, AFP	46.652*
Response to hydrogen peroxide	HBB, HBA2, HPR, HBA1	45.392**
Response to muscle stretch	EDN1, NPPA	44.197*
Hydrogen peroxide catabolic process	HBB, HBA2, HBA1	43.435**
Nitric oxide transport	EDN1, HBB, HBA2, HBA1, AQP1	419.872***

*< .05, **< .01, ***<.001.

neurodegenerative processes and highlights the role of glutamatergic signaling in maintaining synaptic plasticity and cognitive function (17). Regarding OUD, we identified transcriptional regulation, neurodevelopment, and immune-inflammatory processes as key drivers of AA. We also found that positive regulation of DNA-templated transcription, which includes the genes *NIBAN2*, *SOX17*, and *HDAC1,* contributes to transcriptional age-related alterations in OUD. This finding aligns with previous studies on transcriptional dysregulation in aging and highlights the role of histone methylation in this process (18). Concerning StUD, our findings emphasize the role of oxidative stress, hypoxia responses, and cell adhesion pathways. As supported by (18), oxidative stress has an important impact on aging, particularly in the development of chronic diseases like cardiovascular disorders (18).

Table 3. Overview of comparisons between accelerated aging groups (AA+ vs. AA−) and overlaps between groups

	DEG (p < .01, FC <> .5)	GO:BP (p < .05)
SUD	11	6
AUD	463	85
OUD	58	9
StUD	51	17
AUD ∩ OUD	7	2
AUD ∩ StUD	5	6
OUD ∩ StUD	2	1
AUD ∩ OUD ∩ StUD	0	0

This table provides an overview of all comparisons between individuals with accelerated biological aging (AA+) and those without (AA−), including overlaps between SUD groups. Differential gene expression (DEG) analysis was performed for each group. The top rows summarize the number of DEGs and enriched pathways identified in the AA+ versus AA− analyses for each SUD group. The bottom rows present overlaps between SUD subgroups (AUD and OUD; AUD and StUD; OUD and StUD; AUD and OUD and StUD), including DEGs and enriched pathways shared across comparisons.

Our integrative mechanistic analysis identified neuroinflammation, oxidative stress, and mitochondrial dysfunction to be implicated in AA across all SUD subtypes. Mitochondria function is central to maintaining cellular energy homeostasis and regulating oxidative stress responses (15). DEGs such as *NOS3, TXNIP, HTR2A, CSF1, HDAC1, EDN1, THBS1*, and *RELN* are directly implicated in mitochondrial dysfunction and ROS production and can activate the assembly of *NLRP3* through different mechanisms (19–22). The cerebral expression of *NOS3* has been associated with molecular abnormalities related to neurodegeneration, including oxidative stress and mitochondrial dysfunction (19). *TXNIP* overexpression significantly increases mitochondrial complex II activity and promotes the expression of *SDHA*, a subunit of complex II, which is a significant site for reactive oxygen species (ROS) generation (20). ROS production by CSF-1 is crucial for macrophage functions such as pathogen killing, cell signaling, and inflammatory responses (21). *THBS1* activates latent transforming growth factor-beta 1 (TGF-β1), a crucial cytokine involved in inflammation, wound healing, and immune responses, and *THBS1* stimulates the production of ROS through its interaction with *CD47* (23, 24). *HDAC1* can both promote and suppress

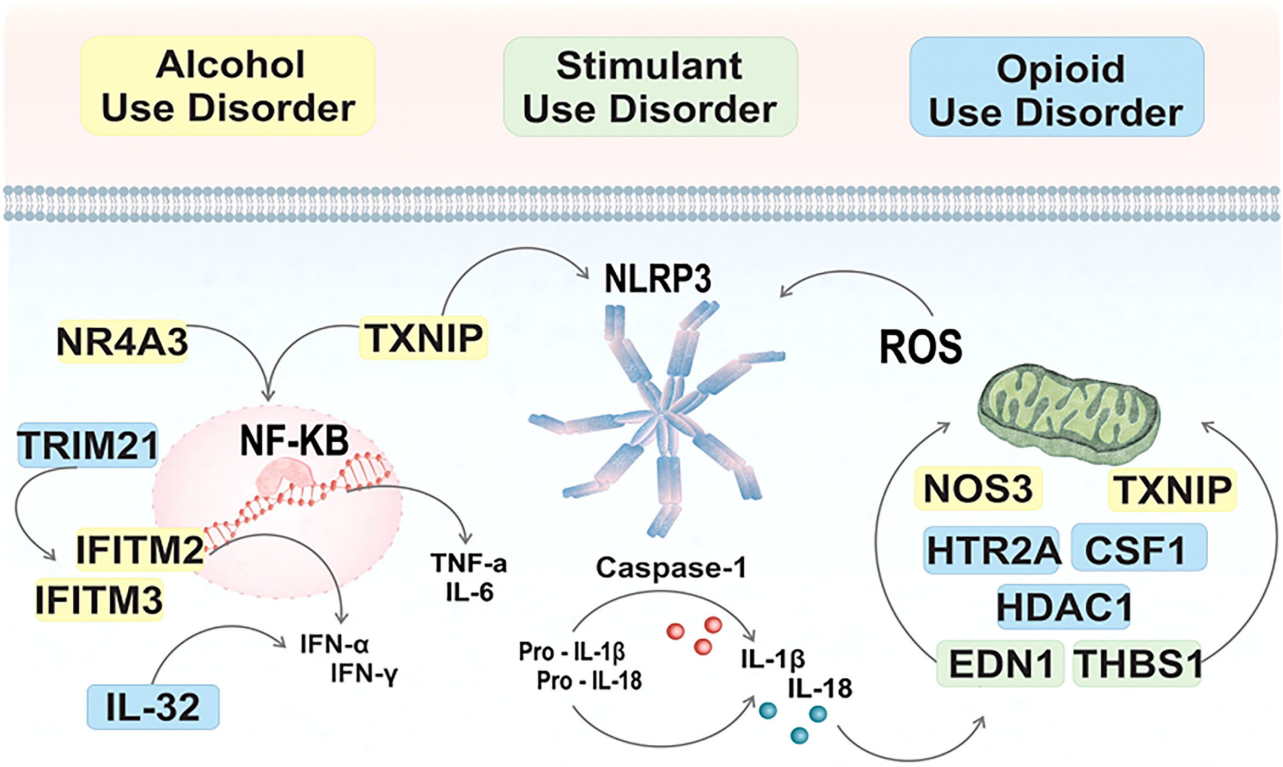

Figure 2. Inflammation and mitochondrial function as mechanisms in AA across SUDs. This figure presents a proposed model linking genes associated with neuroinflammatory and oxidative stress-related pathways across three major SUDs when comparing accelerated aging positive (AA+) and accelerated aging negative (AA−). Genes that are shown in yellow were observed in aging-related pathways within AUD, while the ones in green were observed in aging-related pathways within StUD, and the ones in blue within OUD. The nuclear factor-kappa B (NF-κB) pathway is activated by genes such as *NR4A3*, *TRIM21*, *IFITM2*, *IFITM3*, and *IL-32*, which are involved in inflammatory signaling and immune regulation and might contribute to the production of proinflammatory cytokines (e.g., IFN-α, IFN-γ, TNF-α, IL-6) that may exacerbate neuronal damage. Furthermore, the *TXNIP* and *HDAC1* contribute to inflammasome activation, leading to increased Caspase-1 activity and the subsequent maturation of IL-1β and IL-18, promoting neuroinflammatory responses. Future studies might investigate the role of NLRP3 as a central component in stimulant-induced neuroinflammation in this mechanism. Finally, the upregulation of NOS3, TXNIP, CSF1, HTR2A, HDAC1, EDN1, THBS1, and RELN is linked to vascular dysfunction, cellular stress, and neurodegeneration, might contribute to mitochondrial dysfunction and oxidative stress (ROS).

inflammatory signaling depending on environmental stimuli, which may also influence ROS production (25). *SOX17*, in particular, has been implicated in mitochondrial homeostasis and metabolic regulation, as it influences ATP production, oxidative stress balance, and mitochondrial biogenesis, which are essential for cellular energy metabolism and differentiation (26, 27). Its role in regulating transcription factors such as *HNF1B* and *FOXA2* also highlights its broader impact on mitochondrial function and metabolic adaptation (26).

It is worth mentioning that opposite patterns of regulation were observed in overlapping DEGs for SUD subtypes. For instance, the differential expression of *SOX17* in AUD and OUD may reflect distinct substance-specific effects on cellular stress responses and mitochondrial function in AA+ individuals. In AUD, *SOX17* appears to be upregulated, potentially indicating a compensatory mitochondrial response to alcohol-induced oxidative stress, excitotoxicity, and inflammation (28, 29). In contrast, *SOX17* is downregulated in OUD, which may reflect a blunted or exhausted mitochondrial stress response. As mentioned before, opioids have been shown to impair mitochondrial respiration, increase ROS production, and dysregulate energy metabolism—factors that could lead to suppressed transcriptional regulators like *SOX17*. Another example is NIBAN2, which is upregulated when cells are under stress. We found that *NIBAN2* is upregulated in AUD and downregulated in OUD. In AUD, alcohol-induced oxidative stress may drive the upregulation of *NIBAN2* as a compensatory response to mitigate damage. In contrast, OUD's impact on mitochondrial dysfunction and ROS production may suppress transcriptional responses, leading to the downregulation of *NIBAN2*.

Several limitations of the present study should be acknowledged. The relatively small sample size limits the generalizability of the findings, particularly when subdividing the SUD group into specific subtypes. It is important to emphasize that the SUD subgroups were strictly based on the primary diagnosis determined by the consensus diagnosis process. We excluded participants meeting criteria for any additional SUD diagnoses, as determined from the psychological autopsy. Future studies with larger sample sizes are required to confirm our findings and to further elucidate the mechanisms of AA in different SUDs. The cross-sectional design of this study inherently limits our ability to conclude causality or the temporal progression of AA in individuals with SUD. While we identified associations between molecular profiles and AA status, we cannot determine whether these epigenetic and transcriptomic signatures reflect causal mechanisms, compensatory adaptations, or consequences of long-term substance use. Longitudinal studies that track individuals over time—ideally from active substance use through abstinence or relapse—are essential to disentangle the directionality of these associations and to better understand how biological aging evolves in the context of substance use and related risk factors. Additionally, while our models accounted for several biological and technical covariates, including RNA integrity, tissue pH, smoking index, batch, and estimated cell-type proportions, we acknowledge the likelihood of residual confounding. This limitation is inherent to postmortem studies, where comprehensive individual-level data are often difficult to obtain. Although we conducted detailed psychological autopsy interviews with the donors' next-of-kin and used a rigorous diagnostic consensus process to

determine primary SUD diagnoses, information regarding psychiatric comorbidities, psychotropic medication use, or other medical conditions may be incomplete or inaccurate. These unmeasured factors could influence gene expression and epigenetic profiles and may partially confound our findings. Future studies leveraging larger cohorts and incorporating more detailed clinical records are needed to disentangle these complex relationships. Finally, significant DEGs were identified based on a nominal *p*-value threshold of 0.01 and a fold-change cutoff of 0.5. Notably, no genes survived FDR correction, and thus, all results should be interpreted as exploratory.

In conclusion, this study provides valuable insights into the molecular mechanisms underlying AA in SUD. By identifying genes and enriching biological pathways across various SUDs, we underscore the complexity of substance-induced accelerated epigenetic aging in the brain. Some shared mechanisms of AA between SUD subtypes were noted. Particularly, genes involved in metabolic regulation and mitochondrial function were identified across all disorders. Vascular and oxygen transport system alterations were common in AUD and StUD; cellular signaling, neurodevelopment, and metabolic processes in AUD and OUD; and immune system dysregulation and inflammatory processes in OUD and StUD. Future research should focus on further elucidating these unique aging processes, which may stem from substance-specific molecular signatures or from a combination of factors, such as environmental stressors, comorbidities, and lifestyle influences, that interact with substances use to accelerate biological aging. Understanding these interactions will be critical in developing targeted interventions to mitigate the health risks associated with premature aging in SUD populations.

Materials and Methods

Sample Characteristics and Brain Tissue Samples

Postmortem brain BA9 samples of 62 participants with SUD were obtained from The University of Texas Health Science Center at Houston (UTHealth) Brain Collection, in collaboration with the Harris County Institute of Forensic Science (HCIFS), under the approval of the Institutional Review Board, as described previously (8). For all subjects, informed consent was secured from the next-of-kin and demographic information, autopsy and toxicology reports, and medical and psychiatric notes were obtained if available (8). A structured psychological autopsy interview (30) was conducted with the donor's next-of-kin to obtain detailed information of mental health history, age of onset of drug use, types of substances used, drinking and smoking history, and any co-morbidities. An independent panel of three trained and licensed clinicians reviewed all available information to reach a consensus diagnosis for each subject, classifying them as having a SUD, from which subjects were then categorized into a specific SUD subgroup based on their primary diagnosis.

Four participants ($n = 4$) were excluded following FastQC quality control, and four additional participants ($n = 4$) were removed after being identified as consistent outliers based on Euclidean and Mahalanobis distances in PCA conducted on both cell type proportions and RNA counts. Hence, epigenetic age estimates and clustering analysis (section 2.2, below) were performed for 58 participants with SUD. The Shapiro–Wilk and Kolmogorov–Smirnov tests were used to assess the distribution of variables. Differences in categorical variables were examined using Chi-square tests, while continuous variables were evaluated with Student *t* tests for parametric distributions. Effect sizes were calculated using Cramér's V for Chi-square tests and the r statistic for Student *t* tests.

Epigenetic Clock Estimates and Clustering Analysis

Total DNA extraction and DNAm assays were performed, as described previously (8). Subsequently, DNAm data were processed using the *minfi* and *IlluminaHumanMethylationEPICanno.ilm10b4.hg19* packages (31). IDAT files were imported, and quality control steps included filtering samples with detection *p* values above 0.05 and removing probes with low bead counts. Functional normalization, combined with Noob normalization, was applied to correct for technical noise. Probes failing a detection p-value threshold of 0.01, those associated with SNPs, and those located on sex chromosomes were excluded. Beta values were obtained using the getBeta function, and M-values were obtained using the getM function from *minfi.*

Epigenetic aging was assessed using three clocks specifically designed for brain tissues: $\text{DNAmClock}_{\text{Cortical}}$, $\text{CerebralCortexClock}_{\text{common}}$, and PCBrainAge. $\text{DNAmClock}_{\text{Cortical}}$ was developed to improve the accuracy of age prediction, specifically in human cortex tissue, trained on chronological age using 347 CpG sites relevant to the cerebral cortex (32). The $\text{CerebralCortexClock}_{\text{common}}$ clock was designed to estimate DNAm age specifically for the cerebral cortex, trained using 201 age-associated CpG sites common across different non-cerebellar brain tissues (33). Finally, PCBrainAge was trained using a method of principal component projection on datasets that emphasize brain-specific DNAm patterns associated with Alzheimer's disease (34). $\text{DNAmClock}_{\text{Cortical}}$ and $\text{CerebralCortexClock}_{\text{common}}$ were computed using the *dnaMethyAge* package, while the PCBrainAge was computed using the *calcPCBrainAge* package.

In addition to the brain-specific clocks, we used the PC-Clock package to calculate PCHorvath1, PCHorvath2, PCHannum, PCPhenoAge, and PCGrimAge (35, 36). PCHorvath1 and PCHorvath2 are based on Horvath's original and revised multitissue clocks, respectively, while PCHannum is derived from the Hannum clock, initially trained on blood samples. PCPhenoAge and PCGrimAge are constructed from the PhenoAge and GrimAge clocks, often referred to as "second-generation" clocks, which predict phenotypic aging and mortality risk, respectively.

AA estimates were derived by calculating DNAm-predicted age and regressing this against chronological age, where positive residuals indicate faster-than-expected aging (i.e., AA), and negative residuals indicate slower-than-expected aging (12). To classify subjects into distinct clusters of aging trajectories based on the AA profiles from the three brain-specific clocks, PCA was applied to the standardized AA data to reduce dimensionality and capture the common aging signal across clocks. As PC1 explained 58% of the variance, participants were grouped based on PC1 scores, with positive scores indicating accelerated aging (AA+) and negative scores indicating non-accelerated aging (AA−) (Supplementary Figure S1). Although epigenetic aging is inherently a continuous process, this binary classification was adopted as a pragmatic strategy to enhance interpretability and statistical power in downstream transcriptomic analyses, particularly given the modest sample size. PCA-based grouping allowed us to aggregate the shared signal across partially non-converging clocks, minimizing the noise associated with individual clock variability.

Figure 3 shows the overlap of subjects identified as AA+ or AA− based on each clock, suggesting that although each clock captures distinct aspects of the aging process, there is considerable convergence in identifying individuals with AA in SUD. Correlations within all epigenetic variables, brain epigenetic variables, and chronological age were tested for the entire sample using Pearson tests with the *Hmisc* R package.

Next-generation RNA Sequencing and Differential Expression Analysis

RNA sequencing (RNA-seq) was carried out in BA9 bulk tissue from the same subjects as for DNAm, and data were trimmed for low-quality base pairs and adapter sequences using *trim_galore,* as described previously (36). Sequencing reads were mapped to the human genome build UCSC hg38 using STAR (37), and gene expression was quantified using featureCounts (38). Data was filtered and harmonized with reference gene signatures using curated gene expression profiles from the sigsBrain.rda file (https://rdrr.io/github/unawaz1996/brainyR/man/sigsBrain.html), based on publicly available brain single-cell RNA-seq data (39). The run.DTA function from the *dtangle* package (40) was used to estimate the relative proportions of each cell type, and composite neuronal proportions were calculated by combining excitatory and inhibitory neuron estimates.

Differential expression (DE) analysis was conducted using the R Bioconductor packages *edgeR* (41) and *limma* (42). Sample read counts were filtered to retain only expressed genes, and normalization was performed using the Trimmed Mean of M-values (TMM) method (*calcNormFactors* function in the *edgeR* package). The model matrix was fitted using *lmFit,* and empirical Bayes statistics (*eBayes*) were applied to identify DEGs. DE between AA+ and AA− was assessed for all the SUD samples ($n = 58$) and within each SUD subgroup based on their diagnosis (AUD = 13,

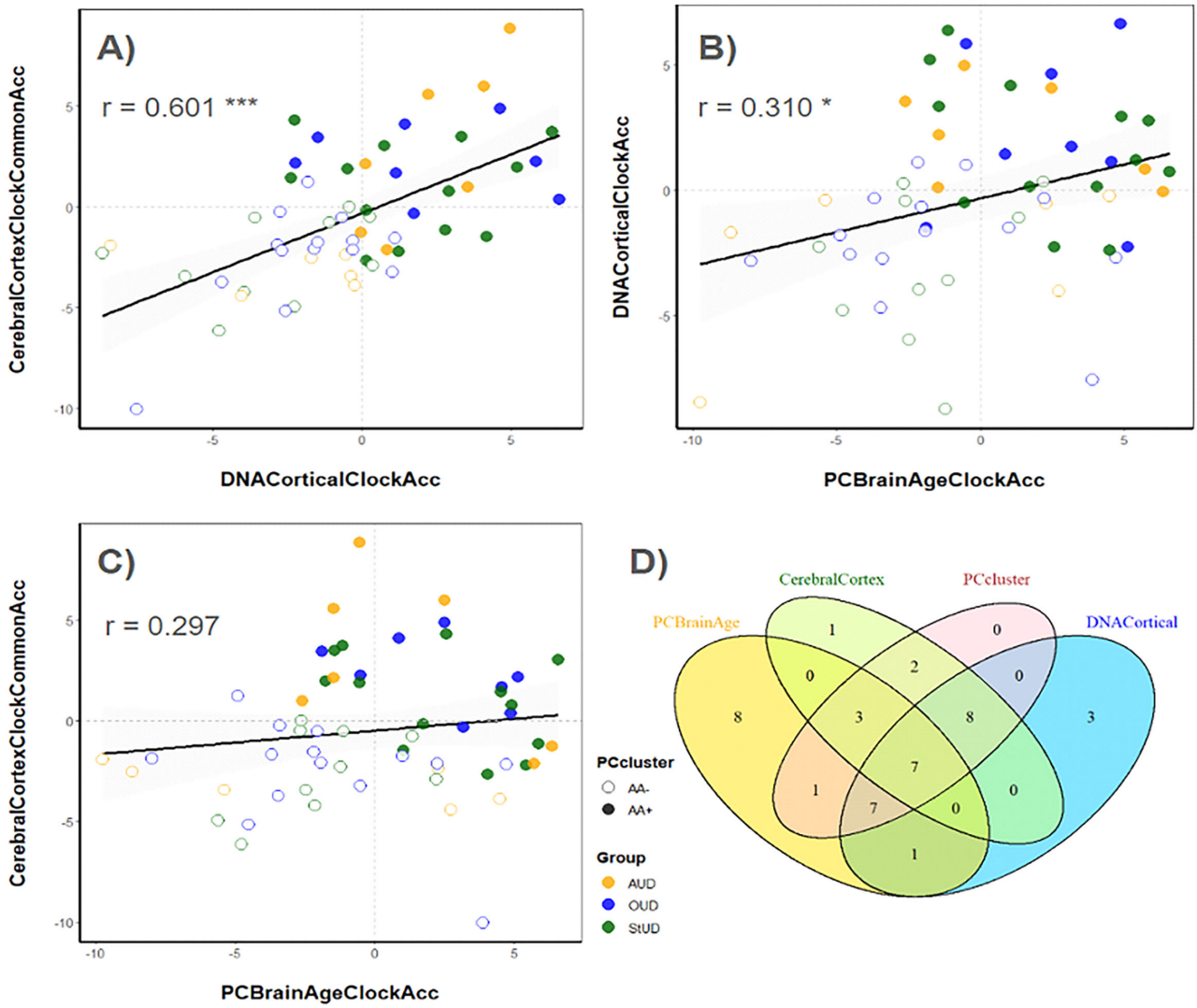

Figure 3. Pearson correlations and Venn diagram of overlaps between aging acceleration based on epigenetic clocks designed for brain tissue. (A–C) Scatter plots showing Pearson correlations between epigenetic aging acceleration measures derived from different brain-specific clocks. Each dot represents a participant, categorized based on their PC cluster classification: accelerated aging positive (AA+, filled dots) or accelerated aging negative (AA−, open circles). (D) Venn Diagram showing overlap of SUD subtypes: AUD (yellow), OUD (blue), and StUD (green). Correlation coefficients (*r*) are annotated for each pair of measures, with significance levels indicated (*$p < .05$; ***$p < .001$). The shaded regions around the regression lines indicate 95% confidence intervals.

OUD = 16, StUD = 10). To minimize confounding effects, SUD subgroups were restricted to participants with a single primary SUD diagnosis, excluding those meeting the criteria for any additional SUD. The models were: ~ *Accelerated Aging [AA+ vs. AA−] + Age [years] + Sex [male vs. female] + Batch [A vs. B] + postmortem interval [PMI in hours] + RNA integrity number [RIN] + tissue pH + smoking index [CpG methylation levels at cg05575921* (43, 44)*] + Astrocytes [proportion].* The proportion of astrocytes was included as a covariate because it accounted for a substantial portion of the variance (16%) in the variance partition analysis (Supplementary Table S1). Significant DEGs were identified based on a nominal *p*-value threshold of 0.01 and a fold change cutoff of 0.5. Results were visualized using EnhancedVolcano, highlighting significant DEGs across conditions. Finally, sensitivity analyses were performed, including individuals with an additional SUD (or secondary diagnosis).

Pathway Analyses

The DEGs were extracted and subjected to enrichment for GO: BP terms. Enrichment analysis was then conducted using the Database for Annotation, Visualization, and Integrated Discovery (DAVID, https://david.ncifcrf.gov) (45), enabling comparison of overlaps between enriched pathways. Significant pathways were identified with a nominal p value ≤ 0.05.

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Data Availability

All data, including DNA methylation data, is available upon request.

Author Contributions

B.K.S. designed and conducted all data analyses and wrote the manuscript. L.S. oversaw processing of all biological samples, DNA methylation, and RNA-seq assays. T.B. contributed to data interpretation and manuscript writing. T.D.M. oversaw all psychological autopsy assessments. The manuscript has been read and approved by all authors. All authors take full responsibility for all data, figures, and text and approve the content and submission of the study. No related work is under consideration elsewhere. All authors state that all unprocessed data are available, and all figures provide accurate presentations of

the original data. Corresponding authors: G.R.F. for conceptualization of epigenetic clock analyses and C.W.B. for overall project conceptualization, postmortem brain collection, and project administration.

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Author Disclosures

The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. All authors have declared no conflict of interest.

References

1. World Health Organization. Global Status Report on Alcohol and Health. Geneva, Switzerland: World Health Organization; 2018, 469. Available from: https://play.google.com/store/books/details?id=qnOyDwAAQBAJ
2. Liu M, Savaskan N, Wang Z, Yang X, Sun Y. Epidemiological Characteristics and Related Risk Factors of the Older Population Aged Over 75 Years. Lausanne, Switzerland: Frontiers Media SA; 2023, 122. Available from: https://books.google.com/books/about/Epidemiological_characteristics_and_rela.html?hl=&id=7yyuEAAAQBAJ
3. Rusanen M, Kivipelto M, Quesenberry CP Jr, Zhou J, Whitmer RA. Heavy smoking in midlife and long-term risk of Alzheimer's disease and vascular dementia. Arch Intern Med. 2011;171(4):333–9. DOI: 10.1001/archinternmed.2010.393. PMID: 20975015
4. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14(10):R115. DOI: 10.1186/gb-2013-14-10-r115. PMID: 24138928; PMCID: PMC4015143
5. Oblak L, van der Zaag J, Higgins-Chen AT, Levine ME, Boks MP. A systematic review of biological, social, and environmental factors associated with epigenetic clock acceleration. Ageing Res Rev. 2021;69:101348. DOI: 10.1016/j.arr.2021.101348. PMID: 33930583
6. Bernabeu E, McCartney DL, Gadd DA, Hillary RF, Lu AT, Murphy L, et al. Refining epigenetic prediction of chronological and biological age. Genome Med. 2023;15(1):12. DOI: 10.1186/s13073-023-01161-y. PMID: 36855161; PMCID: PMC9976489
7. Bachi K, Sierra S, Volkow ND, Goldstein RZ, Alia-Klein N. Is biological aging accelerated in drug addiction? Curr Opin Behav Sci. 2017;13:34–39. DOI: 10.1016/j.cobeha.2016.09.007. PMID: 27774503; PMCID: PMC5068223
8. Cabrera-Mendoza B, Stertz L, Najera K, Selvaraj S, Teixeira AL, Meyer TD, et al. Within subject cross-tissue analyses of epigenetic clocks in substance use disorder postmortem brain and blood. Am J Med Genet B Neuropsychiatr Genet. 2023;192(1-2):13–27. DOI: 10.1002/ajmg.b.32920. PMID: 36056652; PMCID: PMC9742183
9. Luo A, Jung J, Longley M, Rosoff DB, Charlet K, Muench C, et al. Epigenetic aging is accelerated in alcohol use disorder and regulated by genetic variation in APOL2. Neuropsychopharmacology. 2020;45(2):327–36. DOI: 10.1038/s41386-019-0500-y. PMID: 31466081; PMCID: PMC6901591
10. Zindler T, Frieling H, Fliedner L, Veer IM, Neyazi A, Awasthi S, et al. How alcohol makes the epigenetic clock tick faster and the clock reversing the effect of abstinence. Addict Biol. 2022;27(5):e13198. DOI: 10.1111/adb.13198. PMID: 36001430
11. Rosen AD, Robertson KD, Hlady RA, Muench C, Lee J, Philibert R, et al. DNA methylation age is accelerated in alcohol dependence. Transl Psychiatry. 2018;8(1):182. DOI: 10.1038/s41398-018-0233-4. PMID: 30185790; PMCID: PMC6125381
12. Zillich L, Cetin M, Hummel EM, Poisel E, Fries GR, Frank J, et al. Biological aging markers in blood and brain tissue indicate age acceleration in alcohol use disorder. Alcohol Clin Exp Res.2024;48(2):250–9 . DOI: 10.1111/acer.15241. PMID: 38276909; PMCID: PMC10922212
13. Martinez S, Jones JD. A pilot study examining the relationship between chronic heroin use and telomere length among individuals of African ancestry. Pharmacol Biochem Behav. 2023;231:173631. DOI: 10.1016/j.pbb.2023.173631. PMID: 37689117; PMCID: PMC10545475
14. Zhai T, Salmeron BJ, Gu H, Adinoff B, Stein EA, Yang Y. Functional connectivity of dorsolateral prefrontal cortex predicts cocaine relapse: implications for neuromodulation treatment. Brain Commun. 2021;3(2):fcab120. DOI: 10.1093/braincomms/fcab120. PMID: 34189458; PMCID: PMC8226190
15. la Torre A, Lo Vecchio F, Greco A. Epigenetic mechanisms of aging and aging-associated diseases. Cells. 2023;12(8):1163. DOI: 10.3390/cells12081163. PMID: 37190071; PMCID: PMC10136616
16. Carlson ME, Silva HS, Conboy IM. Aging of signal transduction pathways, and pathology. Exp Cell Res. 2008;314(9):1951–61. DOI: 10.1016/j.yexcr.2008.03.017. PMID: 18474281; PMCID: PMC2572856
17. Cox MF, Hascup ER, Bartke A, Hascup KN. Friend or Foe? Defining the role of glutamate in aging and Alzheimer's disease. Front Aging. 2022;3:929474. DOI: 10.3389/fragi.2022.929474. PMID: 35821835; PMCID: PMC9261322
18. Saul D, Kosinsky RL. Epigenetics of aging and aging-associated diseases. Int J Mol Sci. 2021;22(1):401. DOI: 10.3390/ijms22010401. PMID: 33401659; PMCID: PMC7794926
19. de la Monte SM, Jhaveri A, Maron BA, Wands JR. Nitric oxide synthase 3-mediated neurodegeneration after intracerebral gene delivery. J Neuropathol Exp Neurol. 2007;66(4):272–83. DOI: 10.1097/nen.0b013e318040cfa2. PMID: 17413318
20. Li J, Yue Z, Xiong W, Sun P, You K, Wang J. TXNIP overexpression suppresses proliferation and induces apoptosis in SMMC7221 cells through ROS generation and MAPK pathway activation. Oncol Rep. 2017;37(6):3369–76. DOI: 10.3892/or.2017.5577. PMID: 28440491
21. Bhatt NY, Kelley TW, Khramtsov VV, Wang Y, Lam GK, Clanton TL, et al. Macrophage-colony-stimulating factor-induced activation of extracellular-regulated kinase involves phosphatidylinositol 3-kinase and reactive oxygen species in human monocytes. J Immunol. 2002;169(11):6427–34. DOI: 10.4049/jimmunol.169.11.6427. PMID: 12444151
22. Dong F, Zhang X, Wold LE, Ren Q, Zhang Z, Ren J. Endothelin-1 enhances oxidative stress, cell proliferation and reduces apoptosis in human umbilical vein endothelial cells: role of ETB receptor, NADPH oxidase and caveolin-1. Br J Pharmacol. 2005;145(3):323–33. DOI: 10.1038/sj.bjp.0706193. PMID: 15765100; PMCID: PMC1576147
23. Liu Z, Morgan S, Ren J, Wang Q, Annis DS, Mosher DF, et al. Thrombospondin-1 (TSP1) contributes to the development of vascular inflammation by regulating monocytic cell motility in mouse models of abdominal aortic aneurysm. Circ Res. 2015;117(2):129–41. DOI: 10.1161/CIRCRESAHA.117.305262. PMID: 25940549; PMCID: PMC4490953
24. LeBlanc AJ, Kelm NQ. Thrombospondin-1, free radicals, and the coronary microcirculation: the aging conundrum. Antioxid Redox Signal. 2017;27(12):785–801. DOI: 10.1089/ars.2017.7292. PMID: 28762749; PMCID: PMC5647494
25. Dunaway LS, Pollock JS. HDAC1: an environmental sensor regulating endothelial function. Cardiovasc Res. 2022;118(8):1885–903. DOI: 10.1093/cvr/cvab198. PMID: 34264338; PMCID: PMC9239577
26. Jonatan D, Spence JR, Method AM, Kofron M, Sinagoga K, Haataja L, et al. Sox17 regulates insulin secretion in the normal and pathologic mouse β cell. PLoS One. 2014;9(8):e104675. DOI: 10.1371/journal.pone.0104675. PMID: 25144761; PMCID: PMC4140688
27. Lv J, Yi Y, Qi Y, Yan C, Jin W, Meng L, et al. Mitochondrial homeostasis regulates definitive endoderm differentiation of human pluripotent stem cells. Cell Death Discov. 2022;8(1):69. DOI: 10.1038/s41420-022-00867-z. PMID: 35177589; PMCID: PMC8854419
28. Narendra S, Klengel C, Hamzeh B, Patel D, Otten J, Lardenoije R, et al. Genome-wide transcriptomics of the amygdala reveals similar oligodendrocyte-related responses to acute and chronic alcohol drinking in female mice. Transl Psychiatry. 2022;12(1):476. DOI: 10.1038/s41398-022-02231-2. PMID: 36371333; PMCID: PMC9653459
29. Lin SM, Frank DB. SOX17 at the intersection of sex, transcription, and metabolism in pulmonary hypertension. Am J Respir Crit Care Med. 2023;207(8):971–2. DOI: 10.1164/rccm.202302-0284ED. PMID: 36927296; PMCID: PMC10112455
30. Meyer TD, Godfrey CJ, Walss-Bass C. The UT health psychological autopsy interview schedule (UTH-PAIS) – description and reliability of diagnoses and transdiagnostic personality measures. J Psychiatr Res. 2022;156:221–7. DOI: 10.1016/j.jpsychires.2022.10.014. PMID: 36270060
31. Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, et al. Minfi: a flexible and comprehensive bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics. 2014;30(10):1363–9. DOI: 10.1093/bioinformatics/btu049. PMID: 24478339; PMCID: PMC4016708
32. Shireby GL, Davies JP, Francis PT, Burrage J, Walker EM, Neilson GWA, et al. Recalibrating the epigenetic clock: implications for assessing biological age in the human cortex. Brain. 2020;143(12):3763–75. DOI: 10.1093/brain/awaa334. PMID: 33300551; PMCID: PMC7805794
33. Wang Y, Grant OA, Zhai X, Mcdonald-Maier KD, Schalkwyk LC. Insights into ageing rates comparison across tissues from recalibrating cerebellum DNA methylation clock. Geroscience. 2024;46(1):39–56. DOI: 10.1007/s11357-023-00871-w. PMID: 37597113; PMCID: PMC10828477
34. Thrush K, Markov Y, Higgins-Chen A, Morgan L. PCBrainAge: a brain-specific AD-associated DNA methylation clock. Alzheimers Dement. 2022;18:e069230. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/alz.069230
35. Du P, Zhang X, Huang CC, Jafari N, Kibbe WA, Hou L, et al. Comparison of beta-value and M-value methods for quantifying methylation levels by microarray analysis. BMC Bioinformatics. 2010;11:587. DOI: 10.1186/1471-2105-11-587. PMID: 21118553; PMCID: PMC3012676

36. Mendez EF, Wei H, Hu R, Stertz L, Fries GR, Wu X, et al. Angiogenic gene networks are dysregulated in opioid use disorder: evidence from multi-omics and imaging of postmortem human brain. Mol Psychiatry. 2021;26(12):7803–12. DOI: 10.1038/s41380-021-01259-y. PMID: 34385598; PMCID: PMC8837724
37. Dobin A, Gingeras TR. Optimizing RNA-seq mapping with STAR. Methods Mol Biol. 2016;1415:245–62. DOI: 10.1007/978-1-4939-3572-7_13. PMID: 27115637
38. Fdez-Riverola F, Rocha M, Mohamad MS, Caraiman S, Gil-González AB. Practical Applications of Computational Biology and Bioinformatics, 16th International Conference (PACBB 2022). L'Aquila, Italy: Springer Nature; 2022, 122. Available from: https://play.google.com/store/books/details?id=riuWEAAAQBAJ
39. Sutton GJ, Poppe D, Simmons RK, Walsh K, Nawaz U, Lister R, et al. Comprehensive evaluation of deconvolution methods for human brain gene expression. Nat Commun. 2022;13(1):1358. DOI: 10.1038/s41467-022-28655-4. PMID: 35292647; PMCID: PMC8924248
40. Hunt GJ, Freytag S, Bahlo M, Gagnon-Bartsch JA. dtangle: accurate and robust cell type deconvolution. Bioinformatics. 2019;35(12):2093–9. DOI: 10.1093/bioinformatics/bty926. PMID: 30407492
41. Chen Y, Chen L, Lun ATL, Baldoni PL, Smyth GK. edgeR v4: powerful differential analysis of sequencing data with expanded functionality and improved support for small counts and larger datasets. bioRxiv. 2024. DOI: 10.1101/2024.01.21.576131
42. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43(7):e47. DOI: 10.1093/nar/gkv007. PMID: 25605792; PMCID: PMC4402510
43. Gadd DA, Stevenson AJ, Hillary RF, McCartney DL, Wrobel N, McCafferty S, et al. Epigenetic predictors of lifestyle traits applied to the blood and brain. Brain Commun. 2021;3(2):fcab082. DOI: 10.1093/braincomms/fcab082. PMID: 34041477; PMCID: PMC8134833
44. Zillich L, Poisel E, Streit F, Frank J, Fries GR, Foo JC, et al. Epigenetic signatures of smoking in five brain regions. J Pers Med. 2022;12(4):566. DOI: 10.3390/jpm12040566. PMID: 35455681; PMCID: PMC9029407
45. Sherman BT, Panzade G, Imamichi T, Chang W. DAVID ortholog: an integrative tool to enhance functional analysis through orthologs. Bioinformatics. 2024;40(10):btae615. DOI: 10.1093/bioinformatics/btae615. PMID: 39412445; PMCID: PMC11520416

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RESEARCH ARTICLE

Early infant white matter tract microstructure predictors of subsequent change in emotionality and emotional regulation

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There are rapid changes in negative and positive emotionality (NE, PE) and emotional regulation (e.g., soothability) during the first year of life. Understanding the neural basis of these changes during maturation can enhance the understanding of the etiology of early psychopathology. Our goal was to determine how measures of white matter (WM) microstructure in tracts connecting key emotion-related neural networks, including the forceps minor (FM), cingulum bundle (CB), and uncinate fasciculus interconnecting the default mode network (DMN), salience network (SN), and central executive network (CEN), can predict developmental change in infant emotionality and emotional regulation. We used Neurite Orientation Dispersion and Density Imaging (NODDI) measures together with conventional diffusion tensor metrics to examine WM tract microstructure and fiber collinearity in the primary sample ($n = 95$), and modeled each WM feature with caregiver-reported infant NE, PE, and soothability, with infant and caregiver sociodemographic factors as covariates. In 3-month infants, higher neurite dispersion and lower longitudinal fiber alignment in the FM were associated with a larger increase in NE from 3 to 9 months of age, suggesting that greater integration of the DMN, SN, and CEN leads to a larger subsequent increase in NE; while higher neurite density and dispersion as well as lower WM longitudinal alignment in the left CB were associated with a larger increase in PE, suggesting that greater integration within the CEN leads to increasing PE over time. In addition, higher neurite dispersion and lower WM longitudinal alignment in the left CB were associated with a larger increase in soothability. Associations among diffusion tensor measures and changes in infant emotionality and emotional regulation measures were replicated in an independent test sample ($n = 44$). These findings suggest that early infant WM microstructural features support infant emotionality and emotional regulation development and could represent early biomarkers of future emotional and behavioral disorders.

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Keywords: Emotional and behavioral disorder biomarkers, emotionality development, Neurite Orientation Dispersion and Density Imaging (NODDI), infant neuroimaging, white matter

Introduction

Negative and positive forms of emotionality, along with emotional regulation capacities such as soothability, can be reliably assessed in infants within the first months of life. The development of negative emotionality (NE) tends to show relative stability with a trend to increase throughout the first year (1–7); positive emotionality (PE) undergoes rapid increase during this period (8); whereas emotional regulation capacities develop most dramatically in the first few years (9) and continue into adulthood (10). Previous research has shown that these early indices of emotionality and emotional regulation can predict future emotional behavioral outcomes (11–15). For example, high NE is associated with an increased risk for future affective and behavioral disorders (16–22), low PE is linked to a higher risk for future behavioral inhibition and depression (23–28), and low soothability has been linked to future aggression, disruptive behavior, and social engagement problems (29–31). Therefore, identifying objective markers of emotionality and emotional regulation development could provide valuable insights into the etiology of early psychopathology.

White matter (WM) tracts are identifiable early in neonates and undergo rapid development throughout infancy. Several WM tracts connect key regions within large-scale networks that are critical to emotional processing and regulation, including the default mode network (DMN), which supports self-referential processing (32, 33), the salience network (SN), which guides attention toward salient stimuli (33, 34), and the central executive network (CEN), subserving cognitive control (35). These WM tracts include the cingulum bundle (CB), interconnecting prefrontal, cingulate, and parietal cortices, which form connections within and between the DMN and CEN; the uncinate fasciculus (UF), interconnecting prefrontal and anterior temporal structures with the amygdala, and integrating pathways within the DMN and SN; and the forceps minor (FM) of the corpus callosum, interconnecting prefrontal cortical regions, and connecting the DMN, SN, and CEN across hemispheres (36, 37).

Neurite Orientation Dispersion and Density Imaging (NODDI) is a relatively new method of measuring WM tract microstructure. This method uses a multicompartmental model of multishell diffusion MRI (dMRI) that provides higher intracellular specificity than traditional diffusion tensor models by separating intraneuritic and extraneuritic components and free water within a dMRI voxel (38). This method provides estimations of microstructural integrity and myelination using the neurite density index (NDI) and pruning and dispersion using the orientation dispersion index (ODI). Very few studies have examined relationships among NODDI metrics of WM tract microstructure and emotionality or other clinical outcomes in infant, children, or adults. One previous study in young adults showed that first episode psychosis patients had lower NDI in the FM and higher ODI in the UF and FM (39). Furthermore, lower NDI in the FM and CB, along with higher ODI in the CB, were linked with a longer duration of untreated psychosis (39), a dimension of psychopathology characterized by disrupted cognitive and emotional processing. Another study in infants reported that lower 1-month infant UF microstructure, assessed using combined conventional diffusion tensor and NODDI metrics (including NDI, ODI, FA, MD, AD, and RD), was associated with higher 6-month infant fear before correcting for multiple comparisons (40). These findings highlight the potential of using NODDI metrics as proxies for microstructural features in emotion-related WM tracts. Given the above findings linking

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Table 1. Summary of infant-caregiver dyads characteristics for analyses

	Primary		Test	
	3-month Mean ± SD (Min–Max)	9-month Mean ± SD (Min–Max)	3-month Mean ± SD (Min–Max)	9-month Mean ± SD (Min–Max)
Total infant-caregiver pairs	95		44	
Infant				
Age, weeks	14.74 ± 2.72 (10–22)	41.68 ± 4.74 (35–67)	13.59 ± 2.66 (9–19)	39.2 ± 3.14 (36–48)
Biological sex, male/female	56/39		20/24	
Caregiver				
Caregiver age, years	31.80 ± 4.67 (18–42)	—	22.66 ± 1.41 (19–25)	—
Sum of public assistance types	0.94 ± 1.43 (0–5)	—	3.25 ± 1.43 (0–7)	—
EPDS depressed mood	5.27 ± 4.8 (0–22)	5.41 ± 4.52 (0–18)	5.98 ± 5.87 (0–24)	5.55 ± 4.60 (0–22)
PAI BOR affective instability	4.00 ± 3.34 (0–13)	4.32 ± 3.79 (0–16)	6.53 ± 2.81 (0–12)	—
STAI state anxiety	29.16 ± 8.81 (20–61)	27.57 ± 8.45 (20–58)	31.66 ± 9.52 (20–67)	27.89 ± 6.77 (20–50)
STAI trait anxiety	34.01 ± 10.87 (20–69)	33.79 ± 10.64 (21–70)	34.66 ± 8.41 (21–59)	35.05 ± 7.82 (22–55)
Emotional outcomes				
IBQ NE	2.90 ± 0.67 (1.63–4.38)	3.18 ± 0.72 (1.67–5.35)	3.00 ± 0.71 (1.49–4.38)	3.56 ± 0.76 (1.85–5.51)
IBQ PE	3.62 ± 1.27 (1–7)	5.50 ± 0.62 (3.79–6.93)	4.90 ± 1.16 (1.93–6.79)	5.49 ± 0.97 (1.55–6.79)
IBQ soothability	5.41 ± 0.71 (3.71–7)	5.58 ± 0.84 (3.29–7)	5.01 ± 0.78 (3.86–7)	5.12 ± 0.73 (4–6.71)

lower NDI and higher ODI in WM tracts with worse emotional outcomes, it is possible that lower NDI and higher ODI in WM tracts connecting neural regions important for emotional regulation might be associated with higher levels of infant emotionality, especially higher NE. While diffusion tensor imaging has been more commonly used in research to examine WM tract microstructure and fiber collinearity, more research is needed to examine how NODDI and diffusion tensor metrics can be used in infancy to identify indices of WM tract microstructure and fiber collinearity associated with emotional behaviors that represent transdiagnostic risk factors.

We previously reported that lower UF and FM structural integrity measured using normalized quantitative anisotropy, a proxy of directional diffusion, and fractional anisotropy (FA), a proxy measure of WM fiber density in the longitudinal relative to the transverse direction, in 3-month infants predicted greater NE at 9 months (41). Moreover, our recent work demonstrates that greater increases in right UF, FM, and left CB ODI from 3 to 9 months are associated with disrupted development of emotional regulation during the same period, while a greater increase in right UF NDI is linked to a smaller increase in PE in the same timeframe (42). To our knowledge, however, no study has examined the extent to which infant WM tract microstructure predicts developmental changes in emotionality or emotional regulation. The aim of our study was thus to determine the extent to which NODDI indices of WM microstructure predict change in emotionality and emotional regulation from 3 to 9 months of age. Given that early manifestations of emotionality and emotional regulation (43), as well as the onset of neural functional specialization for negative emotion processing (44), are observable in 3-month infants, and emotional dysregulation at 9 months of age can serve as an early indicator of future behavioral and emotional problems (45–49), we chose to study developmental changes in emotionality and emotional regulation within this 3- to 9-month period. Based on the small number of extant findings examining NODDI indices in infancy, we hypothesized that lower NDI and/or higher ODI in the CB, UF, and FM in 3-month-old infants would be associated with a greater increase in NE, a larger decrease or a smaller increase in PE, and/or a larger decrease or a smaller increase in soothability, from 3 to 9 months of age. We used diffusion tensor indices, that is, axial diffusivity (AD) as an indicator of longitudinal fiber alignment, radial diffusivity (RD) as a measure of myelination integrity, and FA to assess WM integrity measured as the balance between axial and radial diffusion, as secondary measures of WM tract microstructure and fiber collinearity to examine relationships among WM measures and changes in emotionality and emotional regulation. We next examined relationships among NODDI and diffusion tensor indices of WM microstructure to determine congruence among the microstructure-emotionality and emotional regulation relationships measured using these different indices. Diffusion tensor measures were then examined in an independent test sample in order to determine the extent to which WM microstructure-emotionality/emotional regulation relationships could be replicated.

To account for external factors that impact WM neurodevelopment (9, 50–57), we included sociodemographic and clinical measures, specifically, caregiver age, and affective states (depression, anxiety, and affective instability), along with infant age and biological sex, as covariates when modeling the relationships between indices of WM tract microstructure and the development of infant emotionality and emotional regulation.

Results

A total of 95 consented infant-caregiver dyads from the primary sample and 44 from the test sample meeting exclusion criteria had usable 3-month dMRI scans. Infant-caregiver dyads characteristics for analyses were summarized in Table 1. Change in infant NE, PE, and soothability from 3 to 9 months are plotted in Figure 1.

Associations Between 3-month WM NODDI Measures and the 3-to-9-month Change in Infant Emotionality

Three-month FM ODI was positively correlated with the 3-to-9-month change in NE ($\beta = 0.334$, $r^2 = 0.112$, $p = 0.0010$, $q = 0.020$; Figure 2A; parameters of models adjusted for covariates in Supplement), indicating that higher FM ODI was associated with a smaller decrease or larger increase in NE. Three-month FM NDI was also positively correlated with the 3-to-9-month change in NE ($\beta = 0.224$, $r^2 = 0.050$, $p = 0.0303$; Supplement Figure S1), but it did not survive the correction for multiple comparisons.

Three-month left CB ODI was positively correlated with the 3-to-9-month change in PE ($\beta = 0.300$, $r^2 = 0.090$, $p = 0.0037$, $q = 0.037$; Figure 2B; Supplement), indicating that higher left CB ODI was associated with a larger increase in PE. Three-month left CB NDI was positively correlated with the 3-to-9-month change in PE ($\beta = 0.283$, $r^2 = 0.080$, $p = 0.0062$, $q = 0.042$; Figure 2C; Supplement), indicating that higher left CB NDI was associated with a larger increase in PE.

Associations Between 3-month WM NODDI Measures and the 3-to-9-month Change in Infant Emotional Regulation

Three-month left CB ODI was positively correlated with the 3-to-9-month change in soothability ($\beta = 0.218$, $r^2 = 0.048$, $p = 0.0369$; Figure 2D; Supplement), indicating that higher left CB ODI was associated with a larger increase in soothability.

Associations Between 3-month WM Diffusion Tensor Measures and the 3-to-9-month Change in Infant Emotionality

Three-month FM AD was negatively correlated with the 3-to-9-month change in NE ($\beta = -0.295$, $r^2 = 0.087$, $p = 0.0039$; Figure 3A), indicating

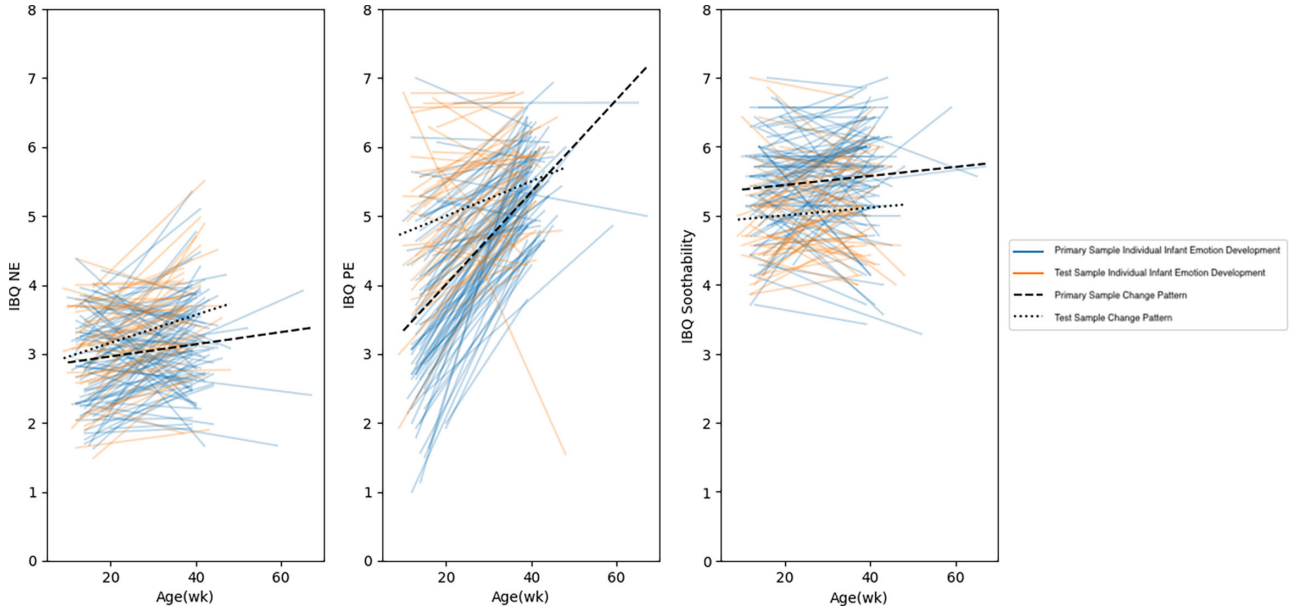

Figure 1. The 3-to-9-month development of infant NE, PE, and soothability.

that lower FM AD was associated with a smaller decrease or a larger increase in NE.

Three-month left CB AD was negatively correlated with the 3-to-9-month change in PE ($\beta = -0.353$, $r^2 = 0.125$, $p = 0.0006$; Figure 3B), indicating that lower left CB AD was associated with a larger increase in PE.

Associations Between 3-month WM Diffusion Tensor Measures and the 3-to-9-month Change in Infant Emotional Regulation

Three-month left CB AD was negatively correlated with the 3-to-9-month change in soothability ($\beta = -0.254$, $r^2 = 0.065$, $p = 0.0144$; Figure 3C), indicating that lower left CB AD was associated with a larger increase in soothability over time.

Correlations Between 3-month NODDI and Diffusion Tensor Measures in WM Tracts in Which Significant Relationships were Shown Among NODDI Measures and Changes in NE, PE, and Soothability

FM ODI was negatively correlated with FM AD ($\rho = -0.843$, $p < 0.0001$), and left CB ODI and NDI were negatively correlated with left CB AD ($\rho = -0.812$, $p < 0.0001$; $\rho = -0.733$, $p < 0.0001$).

Validation of Significant WM Tract Measures—NE, PE, and Soothability Development Relationships

The modeling accuracies in the test sample were: 3-month FM AD – 3-to-9-month NE change root mean square error (RMSE) = 1.488; 3-month left CB AD—3-to-9-month PE change RMSE = 1.027; 3-month left CB AD—3-to-9-month soothability change RMSE = 1.282. These RMSE values reflect good fits of the models in the test sample.

Discussion

In this study, we examined the extent to which early infant WM microstructure may shape changes in emotionality and emotional regulation. Understanding the neural mechanisms underlying these changes can provide neural markers to better predict future behavioral and emotional challenges, as well as informing new intervention strategies and providing objective markers for monitoring response to these interventions. Our main finding for NE development was that higher neurite dispersion in the FM was associated with a smaller decrease or larger increase in NE from 3 to 9 months of age. Regarding PE development, higher neurite density and dispersion in the left CB were associated with a larger increase in PE. These findings indicate that specific microstructural features of WM tracts interconnecting emotion-related neural regions can help predict the subsequent development of emotionality and emotional regulation in infancy.

Greater 3-month neurite dispersion, as indicated by greater ODI, within the FM was significantly associated with a smaller decrease or larger subsequent increase in NE. Greater 3-month FM ODI, a marker of delayed pruning, can lead to greater functional integration of prefrontal cortical regions within the DMN, SN, and CEN. This pattern of greater integration among prefrontal regions across hemispheres at 3 months might then result in an increased influence of the DMN, supporting internalizing and attention to emotionally salient stimuli, on cognitive processes such as executive function supported by the CEN, leading to reduced capacity for emotional regulation. This maladaptive increase in integration across prefrontal regions, parallels our previous findings showing relationships between measures of functional integration among these large-scale neural networks and future depression and mania risk in young adults (58, 59), and children (60), and indicate that these relationships emerge early in infancy.

Greater 3-month microstructural complexity, as indicated by higher NDI and ODI, in the left CB was significantly associated with a larger subsequent increase in PE. Tractography was performed predominantly on the frontoparietal segment of the CB, which connects prefrontal, cingulate, and parietal cortices within the CEN (36, 37), making the anterior part of the CB a major interconnecting bundle of the CEN. One interpretation of this finding is thus that a greater extent of anatomical connectivity and associated functional integration across prefrontal, cingulate, and parietal cortices within the CEN at 3 months can enhance executive function and emotional regulation capacity, resulting in higher levels of PE longer-term. By contrast, lower neurite density and dispersion in the anterior CB at this early age might reduce the ability to process positive emotional experiences and might result in lower levels of PE longer-term. Similarly, greater 3-month left CB ODI was associated with a larger 3-to-9-month increase in soothability, providing further evidence that greater integration within the frontoparietal region of the CB is associated with a greater future capacity for emotional regulation, while lower integration within this region of the CEN at 3 months of age can result in longer-term impairments in emotional regulation capacity.

We previously reported that a greater 3-to-9-month increase in left CB ODI is associated with a greater decrease rather than a greater increase in soothability during the same period (42). Considering these and the present findings together, we hypothesize that as the left CB tract continues to develop during 3 to 9 months of age, there is an anterior to posterior shift in microstructural development of the CB, during which the posterior parietal portion of the CB increasingly integrates the DMN

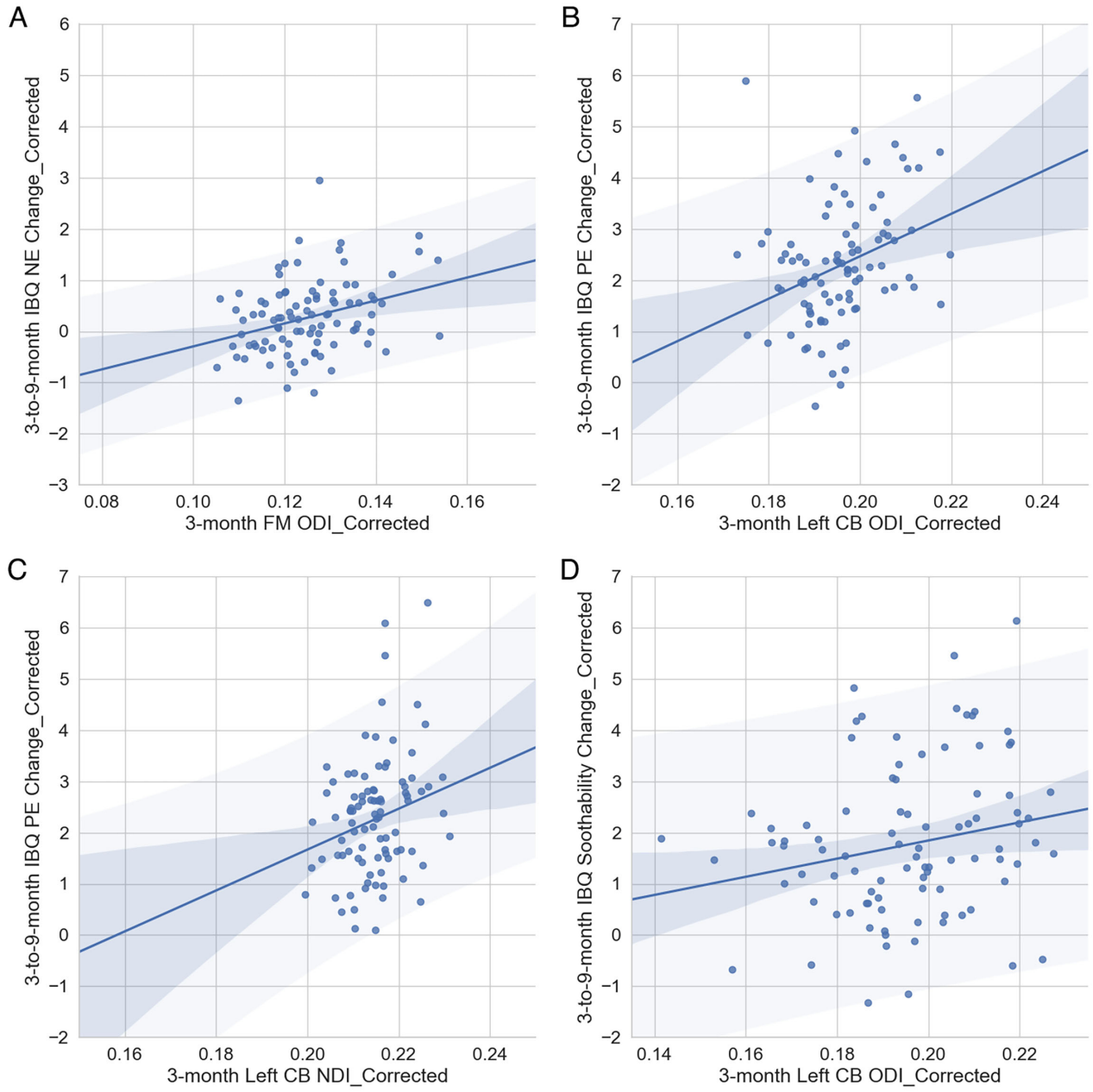

Figure 2. Covariate-corrected relationships between WM NODDI measures and the infant emotionality and emotional regulation development (solid lines as regression lines, brighter shadowed area as prediction interval, and darker shadowed areas as corresponding 95% confidence intervals). (A) Association between 3-month FM ODI and 3-to-9-month NE changes. (B) Association between 3-month left CB ODI and 3-to-9-month PE changes. (C) Association between 3-month left CB NDI and 3-to-9-month PE changes. (D) Association between 3-month left CB ODI and 3-to-9-month soothablity changes.

with the CEN, and other neural networks, resulting in greater interference with emotional regulation capacity. Thus, our findings from the present and this previous study together suggest a nonlinear relationship among CB ODI and emotional regulation capacity during 3 to 9 months of age, whereby greater left CB ODI at 3 months followed by a smaller increase, or greater decrease, in ODI from 3 to 9 months are necessary for the development of higher levels of emotional regulation capacity.

Significant negative correlations were observed among FM ODI and AD, as well as left CB ODI and NDI with AD, suggesting that greater NDI and ODI together might be associated with lower AD. These findings parallel previous reports that ODI may be negatively associated, while NDI may show a smaller but positive correlation, with FA (38). This is because higher ODI, indicating a greater extent of neurite dispersion, is associated with a lower level of longitudinally aligned WM fiber, that is, lower AD and lower AD can contribute to lower FA. Our findings regarding relationships among FM and left CB AD and 3-to-9-month changes in NE, PE and soothability were therefore in the opposite direction from those among FM and left CB ODI and NDI and these emotionality and emotional regulation outcome measures. Furthermore, these AD—outcome measure relationships were replicated in our test sample, indicating the robust nature of these relationships.

We acknowledge several limitations of this study. The sample size of the present study was relatively small with the exclusion of infants who were unable to remain still during scans, and the replication was

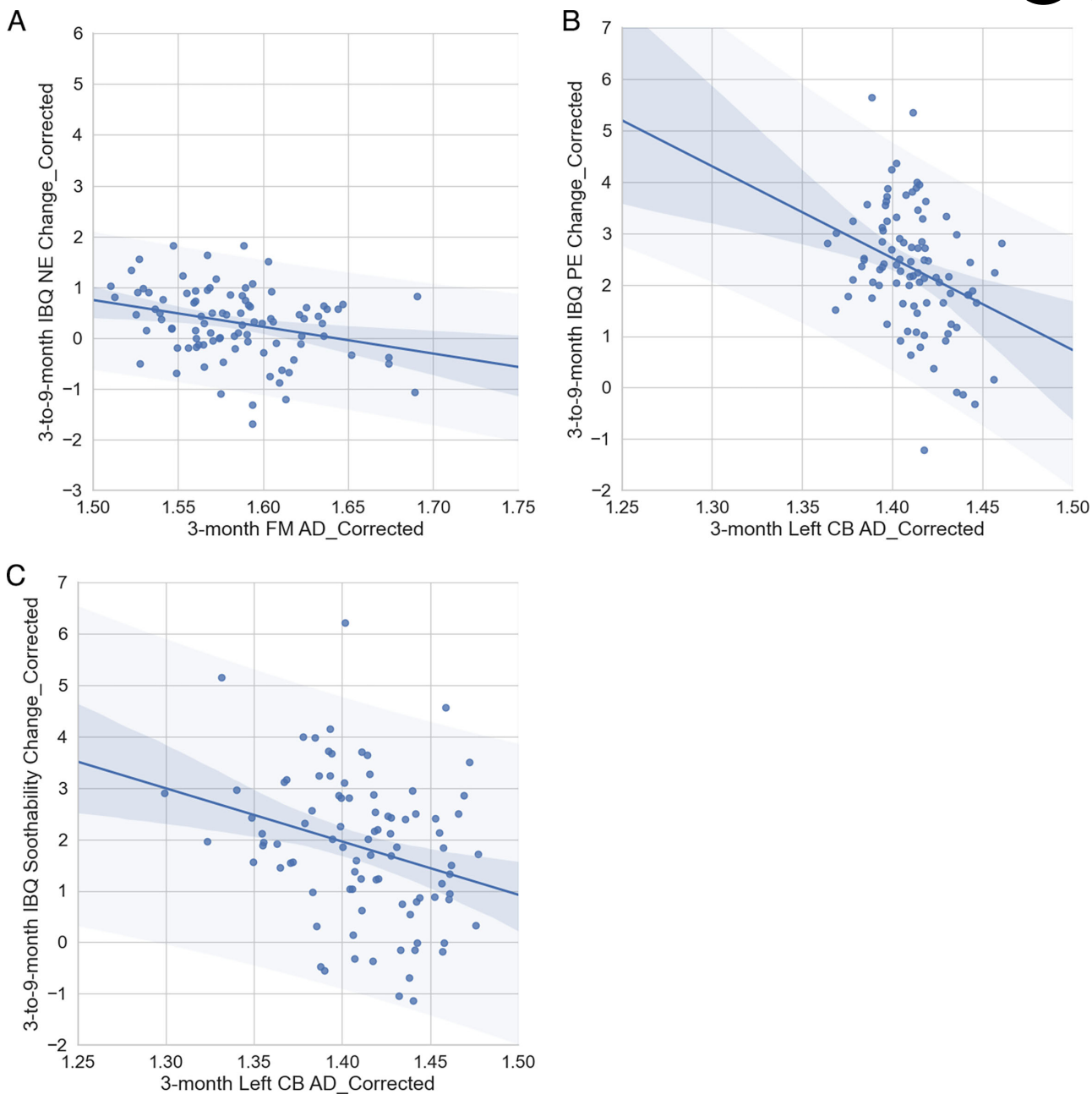

Figure 3. Covariate-corrected relationships between WM diffusion tensor measures and the infant emotionality and emotional regulation development (solid lines as regression lines, brighter shadowed area as prediction interval, and darker shadowed areas as corresponding 95% confidence intervals). (A) Association between 3-month FM AD and 3-to-9-month NE changes. (B) Association between 3-month left CB AD and 3-to-9-month PE changes. (C) Association between 3-month left CB AD and 3-to-9-month soothablity changes.

limited to diffusion metrics. That noted, we were able to replicate our findings in an independent test sample, which is a major strength of the present study. In addition, the test sample in this study was recruited from a higher-risk group, which may have introduced demographic differences between the two samples. However, the high modeling accuracies achieved on the test sample provide further evidence of the consistency of our findings. Future studies aiming to replicate our NODDI metric findings in larger multisite infant imaging cohorts, for example, the HEALthy Brain and Child Development (HBCD) dataset (61), can be performed when these datasets become publicly available. Potential interaction effects between baseline infant emotionality and emotional regulation and caregiver affective states may also be examined with these larger longitudinal datasets. One future direction is to investigate how microstructural features within tracts can predict emotionality and emotional regulation development. Microstructural features extracted from tract subregions may be analyzed in longitudinal infant imaging data to offer insights into tract-specific developmental trajectories, and their relationships with infant emotional behavior.

The present study highlights the important role of FM and left CB ODI and NDI in 3-month-old infants, reflecting integration of critical emotion-related large-scale networks at this age, as predictors of the future development of emotionality and emotional regulation. These insights enhance understanding of the neural mechanisms underlying the development of emotionality and emotional regulation during this critical

developmental period, and provide potential early neural targets to monitor the effectiveness of interventions to mitigate future psychopathology risk.

Materials and Methods

Participants and Measures

The University of Pittsburgh Human Research Protection Office approved all study procedures. Infant-caregiver dyads for the primary sample were identified using three recruitment sources: the University of Pittsburgh Clinical and Translational Science Institute Newborn Research Support Service (NuRSERy) and Community Pediatric Service (Pediatric PittNet), and the University of Pittsburgh Pitt + Me website. The test sample was recruited from the population-based, longitudinal Pittsburgh Girls Study (MH106570). Exclusion criteria for both samples were: (1) infant: preterm birth (<37 weeks postgestational age), low birth weight (<5.5 lb), Apgar score <7 (5 min after birth), abnormal brain morphometry (occipitofrontal circumference <32 cm), extended hospitalization due to physical health problems, and MRI contraindications (pacemakers, aneurysm clips, or non-removable ferromagnetic implants); (2) caregiver: <18 years, prenatal or concurrent illicit substance use (measured via obstetric records or self-report), and <2 h/day care of the infant.

At 3 and 9 months, caregiver report on the Infant Behavior Questionnaire-Revised (IBQ-R) Short Form (1) provided measures of infant NE (i.e., composite of Sadness, Distress to Limitations, Fear, and reverse coded Falling Reactivity/Rate of Recovery from Distress subscales), PE (i.e., composite of Smiling/Laughter and High-Intensity Pleasure subscales), and Soothability. To control for sociodemographic variables that may impact infant brain and/or emotional behaviors infant biological sex and age (weeks) at each research visit, caregiver age (years) and the sum of the types of governmental household public assistance received (a proxy for financial strain) at 3 months were used as covariates. Additionally, caregiver postpartum depression using the Edinburgh Postnatal Depression Scale (EPDS) (62), affective lability using the Personality Assessment Inventory-Borderline Features Scale (PAI-BOR) (63), and state and trait anxiety using the Spielberger State-Trait Anxiety Inventory (STAI) (64) at the 3- and 9-month visits were used as clinical covariates.

Image Acquisition and Processing

MRI scanning procedures were conducted with 3-month-old infants during natural sleep (65) using a 3T Siemens MAGNETOM Skyra MRI system (Siemens Healthcare AG, Erlangen, Germany) with a 32-channel head coil at Children's Hospital of Pittsburgh. Multishell echo planar (EPI) diffusion MRI (dMRI) data were acquired under the following parameters: (1) primary sample: FOV = 200 mm, voxel dimensions = 2.0 × 2.0 × 2.0 mm^3, anterior to posterior phase encoding: TE/TR = 98/2800 ms, 9 reference volumes with b = 0 s/mm^2, 50 volumes with b = 750 s/mm^2 and 100 volumes with 2000 s/mm^2; posterior to anterior phase encoding for EPI distortion correction: TE/TR = 80/2500 ms, 10 reference volumes with b = 0 s/mm^2. (2) Test sample: FOV = 256 mm, voxel dimensions = 2.0 × 2.0 × 2.0 mm^3, TE/TR = 83/10,600 ms, 7 reference volumes with b = 0 s/mm^2, 42 volumes with b = 1000 s/mm^2.

Three-month infant multishell diffusion MRI scans first underwent manual removal of volumes with motion artifacts for quality control, followed by correction for eddy current, motion and EPI distortion with FMRIB Software Library (FSL) 6.0 toolbox's eddy and topup (66, 67). For scans from the primary sample, tissue weight–modulated NODDI metrics were estimated using the NODDI Matlab toolbox following our previous protocol in native space (68). Mean NDI, ODI, FA, AD, and RD were extracted from the forceps minor (FM) and the left and right cingulum bundle (CB) and UF tracts generated using AutoTrack in DSI Studio (version June 7, 2020 build) (Supplemental Table S1) (69, 70). Intracranial volume was based on the brain mask volume. For the test data, tractography of each scan was generated using the same parameter in DSI Studio. FA, AD, and RD maps were harmonized with the primary sample using the neuroComBat (71). Mean harmonized FA, AD, and RD extracted from each WM tract were used for further analysis.

Data Analysis

NODDI (NDI, ODI) measures of each WM tract from the primary sample were modeled with 3-to-9-month changes of infant NE and PE in order to examine relationships among 3-month microstructural features of each WM tract of interest and change in emotionality during this developmental period. Infant and caregiver sociodemographic/clinical variables (i.e., 3- and 9-month infant age in weeks, biological sex, 3-month intracranial volume, 3-month corresponding NE, PE, or soothability baseline; caregiver age, financial strain at 3 months, 3- and 9-month caregiver EPDS, PAI-BOR affective instability, STAI state and trait anxiety) were included as covariates. Multiple comparisons were addressed using false discovery rate for each outcome independently (72). Specifically, 20 comparisons were conducted, corresponding to two NODDI measures for each of five tracts for two outcomes. Soothability was examined as an additional outcome separately from NE and PE, as the 3-to-9-month change in soothability was collinear with the 3-to-9-month change in NE (Supplement Figure S2).

The same modeling approach was applied using diffusion tensor (FA, AD, and RD) measures of WM tract microstructure and fiber collinearity in the primary sample. Correlation analyses were then conducted in the primary sample to examine relationships between 3-month NODDI and diffusion tensor measures, in order to assess the potential congruence of these measures in characterizing WM microstructure.

All significant WM tract index—outcome of interest (change in NE, PE, or soothability) relationships using diffusion tensor measures of WM microstructure and fiber collinearity in the primary sample were then evaluated in the test sample. Here, the modeling accuracy of each significant WM tract index—outcome of interest relationship in the independent test sample was evaluated using the RMSE. Significant relationships among NODDI indices and 3-to-9-month changes in NE, PE and/or soothability in the primary sample were not validated in the independent test sample because the scanning parameters in the latter sample were not optimized for extraction of NODDI indices.

Data Availability

The data analyzed in this study are available upon reasonable request from the corresponding author, subject to applicable regulations and approval.

Author Contributions

Y.Z. conducted image processing, performed data modeling and analysis, wrote the original draft, and reviewed and edited the manuscript. L.B. provided mentorship and oversight, conducted image processing, and reviewed and edited the manuscript. A.V. provided mentorship and reviewed and edited the manuscript. A.S. conducted infant scan visits, managed image storage, and conducted image quality check. M.A. contributed to image processing. M.T. and G.E. conducted infant behavioral and caregiver sociodemographic and clinical data collection. V.J.S. and V.K.L. facilitated study start-up and infant scanning. R.S. managed the data and reviewed and edited the manuscript. H.A. managed the data. A.E.H. acquired funding, provided mentorship, and reviewed and edited the manuscript. M.L.P. acquired funding, provided mentorship, oversight, and resources, and reviewed and edited the manuscript.

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Author Disclosures

The authors have no conflicts of interest related to this work.

References

1. Gartstein MA, Rothbart MK. Studying infant temperament via the revised infant behavior questionnaire. Infant Behav Dev. 2003;26(1):64–86. DOI: 10.1016/j.infbeh.2016.04.001. PMID: 27088863; PMCID: PMC4909571

2. Putnam SP, Gartstein MA, Rothbart MK. Measurement of fine-grained aspects of toddler temperament: the Early Childhood Behavior Questionnaire. Infant Behav Dev. 2006;29(3):386–401. DOI: 10.1016/j.infbeh.2006.01.004. PMID: 17138293; PMCID: PMC4334385
3. Putnam SP, Rothbart MK, Gartstein MA. Homotypic and heterotypic continuity of fine-grained temperament during infancy, toddlerhood, and early childhood. Infant Child Develop. 2008;17(4):387–405. DOI: 10.1002/icd.582.
4. Rothbart MK, Derryberry D, Hershey K. Stability of temperament in childhood: laboratory infant assessment to parent report at seven years. Temperament and Personality Development Across the Life Span. Psychology Press: London, United Kingdom; 2000, 85–119.
5. Arterberry ME, Bornstein MH. Development in Infancy: A Contemporary Introduction. Psychology Press: London, United Kingdom; 2013.
6. Carranza Carnicero JA, Pérez-López J, Del Carmen GonzálezSalinas M, Martínez-Fuentes MT. A longitudinal study of temperament in infancy: stability and convergence of measures. Eur J Pers. 2000;14(1):21–37. DOI: 10.1002/(SICI)1099-0984(200001/02)14:1⟨21::AID-PER367⟩3.0.CO;2-A.
7. Partridge T, Lerner JV. A latent growth-curve approach to difficult temperament. Infant Child Dev. 2007;16(3):255–65. DOI: 10.1002/icd.465.
8. Belsky J, Fish M, Isabella RA. Continuity and discontinuity in infant negative and positive emotionality: Family antecedents and attachment consequences. Dev Psychol. 1991;27(3):421. DOI: 10.1037/0012-1649.27.3.421.
9. Crockenberg SC, Leerkes EM. Infant and maternal behaviors regulate infant reactivity to novelty at 6 months. Dev Psychol. 2004;40(6):1123. DOI: 10.1037/0012-1649.40.6.1123. PMID: 15535761
10. Charles ST, Carstensen LL. Emotion regulation and aging. Handbook of Emotion Regulation, vol. 307. Guilford Press: New York, NY; 2007, 327.
11. Fox NA, Henderson HA, Marshall PJ, Nichols KE, Ghera MM. Behavioral inhibition: linking biology and behavior within a developmental framework. Annu Rev Psychol. 2005;56:235–62. DOI: 10.1146/annurev.psych.55.090902.141532. PMID: 15709935
12. Gladstone GL, Parker GB, Mitchell PB, Wilhelm KA, Malhi GS. Relationship between self-reported childhood behavioral inhibition and lifetime anxiety disorders in a clinical sample. Depress Anxiety. 2005;22(3):103–13. DOI: 10.1002/da.20082. PMID: 16149043
13. Dougherty LR, Klein DN, Durbin CE, Hayden EP, Olino TM. Temperamental positive and negative emotionality and children's depressive symptoms: a longitudinal prospective study from age three to age ten. J Soc Clin Psychol. 2010;29(4):462–88. DOI: 10.1521/jscp.2010.29.4.462.
14. De Pauw SS, Mervielde I. The role of temperament and personality in problem behaviors of children with ADHD. Abnorm Child Psychol. 2011;39:277–91. DOI: 10.1007/s10802-010-9459-1. PMID: 20862537
15. Cicchetti D, Ackerman BP, Izard CE. Emotions and emotion regulation in developmental psychopathology. Dev Psychopathol. 1995;7(1):1–10.
16. Henderson HA, Wachs TD. Temperament theory and the study of cognition–emotion interactions across development. Dev Rev. 2007;27(3):396–427.
17. Sanson A, Hemphill SA, Smart D. Connections between temperament and social development: a review. Soc Dev. 2004;13(1):142–170.
18. Lonigan CJ, Phillips BM, Hooe ES. Relations of positive and negative affectivity to anxiety and depression in children: evidence from a latent variable longitudinal study. J Consult Clin Psychol. 2003;71(3):465–81. DOI: 10.1037/0022-006x.71.3.465. PMID: 12795571
19. Masse LC, Tremblay RE. Behavior of boys in kindergarten and the onset of substance use during adolescence. Arch Gen Psychiatry. 1997;54(1):62–8. DOI: 10.1001/archpsyc.1997.01830130068014. PMID: 9006402
20. Vitaro F, Barker ED, Boivin M, Brendgen M, Tremblay RE. Do early difficult temperament and harsh parenting differentially predict reactive and proactive aggression? J Abnorm Child Psychol. 2006;34:681–91. DOI: 10.1007/s10802-006-9055-6. PMID: 17048109
21. Eisenberg N, Valiente C, Spinrad TL, Cumberland A, Liew J, Reiser M, et al. Longitudinal relations of children's effortful control, impulsivity, and negative emotionality to their externalizing, internalizing, and co-occurring behavior problems. Dev Psychol. 2009;45(4):988. DOI: 10.1037/a0016213. PMID: 19586175; PMCID: PMC2775424
22. Slutske WS, Moffitt TE, Poulton R, Caspi A. Undercontrolled temperament at age 3 predicts disordered gambling at age 32: a longitudinal study of a complete birth cohort. Psychol Sci. 2012;23(5):510–6. DOI: 10.1177/0956797611429708. PMID: 22457426; PMCID: PMC3556900
23. Tellegen A. Structures of mood and personality and their relevance to assessing anxiety, with an emphasis on self-report. Anxiety and the Anxiety Disorders. Lawrence Erlbaum Associates, Inc: Hillsdale, NJ, US; 1985, 681–706.
24. Watson D, Wiese D, Vaidya J, Tellegen A. The two general activation systems of affect: structural findings, evolutionary considerations, and psychobiological evidence. J Pers Soc Psychol. 1999;76(5):820.
25. Park S-Y, Belsky J, Putnam S, Crnic K. Infant emotionality, parenting, and 3-year inhibition: exploring stability and lawful discontinuity in a male sample. Dev Psychol. 1997;33(2):218. DOI: 10.1037//0012-1649.33.2.218. PMID: 9147831
26. Shankman SA, Tenke CE, Bruder GE, Durbin CE, Hayden EP, Klein DN. Low positive emotionality in young children: association with EEG asymmetry. Dev Psychopathol. 2005;17(1):85–98. DOI: 10.1017/s0954579405050054. PMID: 15971761
27. Oldehinkel AJ, Hartman CA, De Winter AF, Veenstra R, Ormel J. Temperament profiles associated with internalizing and externalizing problems in preadolescence. Dev Psychopathol. 2004;16(2):421–40. DOI: 10.1017/s0954579404044591. PMID: 15487604
28. Hayden EP, Klein DN, Durbin CE, Olino TM. Positive emotionality at age 3 predicts cognitive styles in 7-year-old children. Dev Psychopathol. 2006;18(2):409–23. DOI: 10.1017/S0954579406060226. PMID: 16600061
29. Crockenberg SC, Leerkes EM, Bárrig Jó PS. Predicting aggressive behavior in the third year from infant reactivity and regulation as moderated by maternal behavior. Dev Psychopathol. 2008;20(1):37–54. DOI: 10.1017/S0954579408000023. PMID: 18211727
30. Kochanska G, Tjebkes JL, Fortnan DR. Children's emerging regulation of conduct: restraint, compliance, and internalization from infancy to the second year. Child Dev. 1998;69(5):1378–89. PMID: 9839422
31. Baumeister RF, Vohs KD. Handbook of Self-regulation. Guilford Press: New York; 2004.
32. Spreng RN, Mar RA, Kim AS. The common neural basis of autobiographical memory, prospection, navigation, theory of mind, and the default mode: a quantitative meta-analysis. J Cogn Neurosci. 2009;21(3):489–510. DOI: 10.1162/jocn.2008.21029. PMID: 18510452
33. Menon V. Developmental pathways to functional brain networks: emerging principles. Trends Cogn Sci. 2013;17(12):627–40. DOI: 10.1016/j.tics.2013.09.015. PMID: 24183779
34. Menon V, Uddin LQ. Saliency, switching, attention and control: a network model of insula function. Brain Struct Funct. 2010;214(5-6):655–67. DOI: 10.1007/s00429-010-0262-0. PMID: 20512370; PMCID: PMC2899886
35. Dosenbach NU, Fair DA, Cohen AL, Schlaggar BL, Petersen SE. A dual-networks architecture of top-down control. Trends Cogn Sci. 2008;12(3):99–105. DOI: 10.1016/j.tics.2008.01.001. PMID: 18262825; PMCID: PMC3632449
36. Catani M, De Schotten MT. A diffusion tensor imaging tractography atlas for virtual in vivo dissections. Cortex. 2008;44(8):1105–32. DOI: 10.1016/j.cortex.2008.05.004. PMID: 18619589
37. Nozais V, Forkel SJ, Petit L, Talozzi L, Corbetta M, Thiebaut de Schotten M, et al. Atlasing white matter and grey matter joint contributions to resting-state networks in the human brain. Commun Biol. 2023;6(1):726. DOI: 10.1038/s42003-023-05107-3. PMID: 37452124; PMCID: PMC10349117
38. Zhang H, Schneider T, Wheeler-Kingshott CA, Alexander DC. NODDI: practical in vivo neurite orientation dispersion and density imaging of the human brain. Neuroimage. 2012;61(4):1000–16. DOI: 10.1016/j.neuroimage.2012.03.072. PMID: 22484410
39. Kraguljac NV, Monroe WS, Anthony T, Jindal RD, Hill H, Lahti AC. Neurite orientation dispersion and density imaging (NODDI) and duration of untreated psychosis in antipsychotic medication-naïve first episode psychosis patients. Neuroimage Rep. 2021;1(1):100005. DOI: 10.1016/j.ynirp.2021.100005. PMID: 36969709; PMCID: PMC10038586
40. Planalp EM, Dowe KN, Alexander AL, Goldsmith HH, Davidson RJ, Dean DC III. White matter microstructure predicts individual differences in infant fear (but not anger and sadness). Dev Sci. 2023;26(3):e13340. DOI: 10.1111/desc.13340. PMID: 36367143; PMCID: PMC10079554
41. Banihashemi L, Bertocci MA, Alkhars HM, Versace A, Northrup JB, Lee VK, et al. Limbic white matter structural integrity at 3 months prospectively predicts negative emotionality in 9-month-old infants: a preliminary study. J Affect Disord. 2020;273:538–41. DOI: 10.1016/j.jad.2020.04.029. PMID: 32560951; PMCID: PMC7367553
42. Zhang Y, Banihashemi L, Versace A, Samolyk A, Abdelkader M, Taylor M, et al. Associations among white matter microstructural changes and the development of emotional reactivity and regulation in infancy. Mol Psychiatry. 2025. DOI: 10.1038/s41380-025-03025-w
43. Feldman R, Greenbaum CW, Yirmiya N, Mayes LC. Relations between cyclicity and regulation in mother-infant interaction at 3 and 9 months and cognition at 2 years. J App Develop Psychol. 1996;17(3):347–65.
44. Blasi A, Mercure E, Lloyd-Fox S, Thomson A, Brammer M, Sauter D, et al. Early specialization for voice and emotion processing in the infant brain. Curr Biol. 2011;21(14):1220–4. DOI: 10.1016/j.cub.2011.06.009. PMID: 21723130
45. Stifter CA, Jain A. Psychophysiological correlates of infant temperament: stability of behavior and autonomic patterning from 5 to 18 months. Dev Psychobiol. 1996;29(4):379–91. DOI: 10.1002/(SICI)1098-2302(199605)29:4⟨379::AID-DEV5⟩3.0.CO;2-N. PMID: 8732809

46. Martin RP, Wisenbaker J, Baker J, Huttunen MO. Gender differences in temperament at six months and five years. Infant Behav Develop. 1997;20(3):339–47.
47. Bates JE, Bayles K, Bennett DS, Ridge B, Brown MM. Origins of externalizing behavior problems at eight years of age. In: Pepler Debra J., Rubin KH, editors. The Development and Treatment of Childhood Aggression, vol. 17. Lawrence Erlbaum Associates: Hillsdale, NJ; 1991, pp 93–120.
48. Teerikangas OM, Aronen ET, Martin RP, Huttunen MO. Effects of infant temperament and early intervention on the psychiatric symptoms of adolescents. J Am Acad Child Adolesc Psychiatry. 1998;37(10):1070–6. DOI: 10.1097/00004583-199810000-00017. PMID: 9785718
49. Moore GA, Cohn JF, Campbell SB. Infant affective responses to mother's still face at 6 months differentially predict externalizing and internalizing behaviors at 18 months. Dev Psychol. 2001;37(5):706–14. PMID: 11552765
50. Britton JR. Infant temperament and maternal anxiety and depressed mood in the early postpartum period. Women Health. 2011;51(1):55–71. DOI: 10.1080/03630242.2011.540741. PMID: 21391161
51. Feldman R, Granat A, Pariente C, Kanety H, Kuint J, Gilboa-Schechtman E. Maternal depression and anxiety across the postpartum year and infant social engagement, fear regulation, and stress reactivity. J Am Acad Child Adolesc Psychiatry. 2009;48(9):919–27. DOI: 10.1097/CHI.0b013e3181b21651. PMID: 19625979
52. Hane AA, Fox NA. Ordinary variations in maternal caregiving influence human infants' stress reactivity. Psychol Sci. 2006;17(6):550–6. DOI: 10.1111/j.1467-9280.2006.01742.x. PMID: 16771807
53. Tronick E, Reck C. Infants of depressed mothers. Harv Rev Psychiatry. 2009;17(2):147–56. DOI: 10.1080/10673220902899714. PMID: 19373622
54. Hobson RP, Patrick M, Crandell L, García–Pérez R, Lee A. Personal relatedness and attachment in infants of mothers with borderline personality disorder. Dev Psychopathol. 2005;17(2):329–47. DOI: 10.1017/s0954579405050169. PMID: 16761548
55. Crandell LE, Patrick MP, Hobson RP. 'Still-face' interactions between mothers with borderline personality disorder and their 2-month-old infants. Br J Psychiatry. 2003;183(3):239–47. DOI: 10.1192/bjp.183.3.239. PMID: 12948998
56. Apter G, Devouche E, Garez V, Valente M, Genet M-C, Gratier M, et al. The still-face: a greater challenge for infants of mothers with borderline personality disorder. J Pers Disord. 2017;31(2):156–69. DOI: 10.1521/pedi_2016_30_243. PMID: 27088165
57. Gratz KL, Kiel EJ, Latzman RD, Elkin TD, Moore SA, Tull MT. Emotion: empirical contribution: maternal borderline personality pathology and infant emotion regulation: examining the influence of maternal emotion-related difficulties and infant attachment. J Pers Disord. 2014;28(1):52–69. DOI: 10.1521/pedi.2014.28.1.52. PMID: 24344887
58. Bertocci MA, Afriyie-Agyemang Y, Rozovsky R, Iyengar S, Stiffler R, Aslam HA, et al. Altered patterns of central executive, default mode and salience network activity and connectivity are associated with current and future depression risk in two independent young adult samples. Mol Psychiatry. 2023;28(3):1046–56. DOI: 10.1038/s41380-022-01899-8. PMID: 36481935; PMCID: PMC10530634
59. Schumer MC, Bertocci MA, Aslam HA, Graur S, Bebko G, Stiffler RS, et al. Patterns of neural network functional connectivity associated with mania/hypomania and depression risk in 3 independent young adult samples. JAMA Psychiatry. 2024;81(2):167–77. DOI: 10.1001/jamapsychiatry.2023.4150. PMID: 37910117; PMCID: PMC10620679
60. Fournier JC, Bertocci M, Ladouceur CD, Bonar L, Monk K, Abdul-Waalee H, et al. Neural function during emotion regulation and future depressive symptoms in youth at risk for affective disorders. Neuropsychopharmacology. 2021;46(7):1340–7. DOI: 10.1038/s41386-021-01001-w. PMID: 33782511; PMCID: PMC8134479
61. Jordan CJ, Weiss SR, Howlett KD, Freund MP. Introduction to the special issue on "Informing longitudinal studies on the effects of maternal stress and substance use on child development: planning for the HEALthy brain and child development (HBCD) study". Adver Resil Sci. 2020;1(4):217–21. DOI: 10.1007/s42844-020-00022-6. PMID: 33106790; PMCID: PMC7578232
62. Cox J, Holden J. Perinatal Mental Health: A Guide to the Edinburgh Postnatal Depression Scale (EPDS). Royal College of Psychiatrists: London, United Kingdom; 2003.
63. Trull TJ. Borderline personality disorder features in nonclinical young adults: 1. Identification and validation. Psychol Assess. 1995;7(1):33–41.
64. Spielberger CD. Test anxiety inventory. Palo Alto, CA: Consulting Psychologists Press; 1980.
65. Haney B, Reavey D, Atchison L, Poull J, Dryer L, Anderson B, et al. Magnetic resonance imaging studies without sedation in the neonatal intensive care unit: safe and efficient. J Perinat Neonatal Nurs. 2010;24(3):256–66. DOI: 10.1097/JPN.0b013e3181e8d566. PMID: 20697244
66. Andersson JL, Skare S, Ashburner J. How to correct susceptibility distortions in spin-echo echo-planar images: application to diffusion tensor imaging. Neuroimage. 2003;20(2):870–88. DOI: 10.1016/S1053-8119(03)00336-7. PMID: 14568458
67. Andersson JL, Sotiropoulos SN. An integrated approach to correction for off-resonance effects and subject movement in diffusion MR imaging. Neuroimage. 2016;125:1063–78. DOI: 10.1016/j.neuroimage.2015.10.019. PMID: 26481672; PMCID: PMC4692656
68. Zhang Y, Banihashemi L, Versace A, Samolyk A, Taylor M, English G, et al. Early infant prefrontal cortical microstructure predicts present and future emotionality. Biol Psychiatry. 2024;96(12):959–70. DOI: 10.1016/j.biopsych.2024.04.001. PMID: 38604525; PMCID: PMC11461701
69. Yeh F-C, Verstynen TD, Wang Y, Fernández-Miranda JC, Tseng W-YT. Deterministic diffusion fiber tracking improved by quantitative anisotropy. PLoS One. 2013;8(11):e80713. DOI: 10.1371/journal.pone.0080713. PMID: 24348913; PMCID: PMC3858183
70. Banihashemi L, Schmithorst VJ, Bertocci MA, Samolyk A, Zhang Y, Santos JPL, et al. Neural network functional interactions mediate or suppress white matter–Emotional behavior relationships in infants. Biol Psychiatry. 2023;94(1):57–67. DOI: 10.1016/j.biopsych.2023.03.004. PMID: 36918062; PMCID: PMC10365319
71. Fortin J-P, Cullen N, Sheline YI, Taylor WD, Aselcioglu I, Cook PA, et al. Harmonization of cortical thickness measurements across scanners and sites. Neuroimage. 2018;167:104–20. DOI: 10.1016/j.neuroimage.2017.11.024. PMID: 29155184; PMCID: PMC5845848
72. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol. 1995;57(1):289–300.

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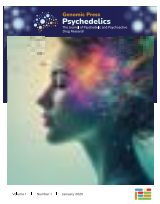

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