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Stem Cell Differentiation in Kidney, Heart, and Lung Organoids: A Comprehensive Review

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Abstract

Organ transplantation, specifically that of the heart, lung, and kidney, is vital to the survival and quality of life for thousands of Americans. However, limited availability and viability of human-derived organ transplants threatens the health of many. Organoids serve as a novel solution to this issue, however the current lack of uniform understanding limits further application. This review focuses on synthesizing the components of heart, lung, and kidney organoid development to provide a clearer comprehension for optimization of future research. Stem cells are the basis of organoid development, and the usage of human pluripotent stem cells (HPSCs) is common to all three organoid types. Differentiation is the avenue by which stem cells become functional organoids. This process consists of the same general elements for all organoid types, comprising a tissue-specific culture media, favoring a 3D culture structure for more accurate morphological resemblance, and resulting in an organoid with functional and structural similarities to their native organ counterpart. These organoids are currently utilized to model disease, study organ development, and test drugs, but limitations in development prevent further implementation into clinical applications. The limitation common to all three organoid types is the lack of uniform differentiation process, resulting in limited reproducibility and replicability. Ultimately, by overcoming such limitations and garnering a greater understanding of organoid development, research can be further developed, enabling organoids to serve as the future of regenerative medicine, transplantation, and human health as a whole.

Keywords: Organoids, stem cells, differentiation, regenerative medicine

Organ transplantation is the most vital procedure for patients in life-threatening conditions, saving and improving the quality of life for nearly 50,000 Americans each year ("Organ Donation Statistics," 2025). Those impacted by chronic illnesses, such as end-stage renal disease and coronary artery disease, are given an opportunity to live a longer and healthier life. Despite this, an extreme disparity exists between the need for organ transplants compared to the availability and viability of organs ("Lung Transplant," n.d.). The kidneys, heart, and lung are the most impacted by such constraints ("Organ Donation Statistics," 2025). For example, in September 2024, nearly 90,000 patients were on the waiting list for a kidney transplant throughout the United States ("Organ Donation Statistics," 2025). However, only 27,332 received kidneys via organ transplant in 2023 ("Organ Donation Statistics," 2025). This vast inconsistency remains prevalent among these organs. As traditional organ transplants prove unreliable to many patients across the United States, an alternative method prevails: organoid development.

Organoids, named for resembling organs, are small masses of cells that are artificially grown to replicate the function and structure of specific organs utilizing stem cells cultivated from them (Yang, 2023). Scientists and researchers have developed organoids that serve to compensate for the function of the heart, lung, and kidneys, implementing them into therapeutic and clinical procedures as a replacement for natural organ transplantation (Sahara, 2023). This development process varies widely and is heavily dependent on the intended use of the organoid that is being cultivated, resulting in a diverse range of successes coupled with limitations (Yang, 2023). Stem cell differentiation is the key factor that elicits this difference.

Stem Cells

Stem cells are highly specialized cells that possess the unique ability to self-renew and differentiate into any cell type, making them incredibly versatile (Poliwoda, 2022). Their multifaceted nature enables stem cells to be the basis for the creation and development of organoid models of the kidneys, heart, and lungs (Yang, 2023). Although stem cells are the basis of organoid creation, there are distinct differences in the types of stem cells used, which are often specific to the organoid that is being created (National Research Council (US), 2002).

Human pluripotent stem cells (HPSCs) are a type of stem cell that can differentiate into any cell within the human body (Zhu Z, Huangfu D, 2013). These stem cells are utilized in the development of kidney, heart, and lung organoids (Morizane, 2015). HPSCs encompass both human embryonic stem cells and induced human pluripotent stem cells (Narsinh, 2011). Given their ability to indefinitely self-renew within a culture while also remaining transformable into any human cell type, HPSCs are prominent in the field of developmental biology (Zhu Z, Huangfu D, 2013). This is especially advantageous in the development of organoids, as they facilitate the creation of masses of cells that resemble functioning organs (Morizane, 2015). For this tremendous quality, HPSCs are used in the creation of all aforementioned organoids (Jacob, 2017).

Embryonic stem cells (ESCs) are a type of HPSC derived from the inner cell mass of blastocysts, or early-stage embryos (National Research Council (US), 2002). These stem cells can continuously self-renew and have the ability to differentiate into specialized cell types, forming masses of cells that can contain tissues such as epithelium, muscle, bone, lung tissue, and neural cells (Narsinh, 2011). They are widely used in regenerative medicine, serving as an important component for the development of all organoids (Poliwoda, 2022).

Induced pluripotent stem cells (iPSCs) are also a type of HPSC (Narsinh, 2011). They are similar to ESCs as they possess the same capacity to differentiate into all specialized cell types, however iPSCs are derived from somatic cells instead of blastocysts (Narsinh, 2011). Since somatic cells are not pluripotent, they require reprogramming through the overexpression of certain transcription factors to become differentiable into many cell types (Narsinh, 2011). iPSCs can be derived from most adult somatic cell types, enabling patients to use their own somatic cells for therapeutic purposes, limiting rejection that may occur from using ESCs, which are less specific (Narsinh, 2011).

Additionally, the endogenous stem cell is a type of stem cell that is tissue-specific and has the ability to self-renew as well as differentiate into different cell types (Xia, 2018). For example, endogenous stem cells are found within the adult lung in specific niches, including the bronchoalveolar junction (Parekh, 2020). These stem cells carry the purpose of repairing damage to the lung and maintaining lung tissue as well as function (Xia, 2018). The use of endogenous stem cells is frequently utilized in the development of organoids of the lung (Xia, 2018).

Ultimately, stem cells are the foundation that enable the development of such biological models and are crucial to the advancements to be made in this field. Due to their general ability to regenerate and differentiate into various cell types, it is essential to understand the distinct properties of particular stem cells in order to optimally harness their capabilities (Poliwoda, 2022).

Differentiation

Cell differentiation is the process by which an unspecialized cell becomes specialized and develops into mature cells with distinct structures and functions within the body (Morizane, 2015). This biological process enables organoid generation, as the aforementioned stem cells are differentiated into specific structures of the kidney, heart, or lungs to form organoids (Yang, 2023). Cell differentiation takes a different shape for each individual organoid, as factors including media, culture structure, and transcription markers result in characteristics unique to the respective organoid (Yang, 2023).

The creation of kidney organoid models begins with the differentiation of human pluripotent stem cells (HPSCs) into multipotent nephron progenitor cells (NPCs) (Morizane, 2015). These NPCs are able to form nephron-like structures, thus serving as the foundation for kidney organoids (Morizane, 2015). During this differentiation process, HPSCs are placed in a culture media with BMP4 and CHIR (Morizane, 2015). BMP4, or bone morphogenetic protein 4, is crucial for cell differentiation and development (Shore, 1998). It is particularly involved in mesenchymal stem cell differentiation, and they help influence the function of NPCs (Morizane, 2015). CHIR is a small molecular inhibitor popularly used in organoid development as it promotes self-renewal and the efficiency of stem cell isolation (Kerr, 2023). Working in conjunction with the BMP4 in the culture media, CHIR enhances the transdifferentiation of mesenchymal stem cells important for the formation of typical kidney structures (Kerr, 2023). Kidney organoids can be grown in both two-dimensional and three-dimensional cultures without any significant differences in function (Morizane, 2015). Two-dimensional (2D) cell cultures are thin and flat, meaning they are easy to use and develop but do not mimic native organ structure (Morizane, 2015). Contrarily, three-dimensional (3D) cell cultures more closely resemble in vivo structures, and therefore, are more accurate for organ modeling (Morizane, 2015). Following differentiation, NPCs exhibit genes and transcription factors such as SIX2, SALL1, WT1, and PAX2 (Morizane, 2015). Each of these carries a specific function in kidney organoid development, aligning with the genes involved in natural kidney development (Morizane, 2015). These NPCs can further form into renal vesicles with additional kidney developmental genes and transcription factors, harboring the ability to self-differentiate into nephron-like structures including loops of Henle, proximal tubules, and glomeruli which resemble the arrangement of nephrons (Morizane, 2015). Ultimately, this differentiation process results in an organoid model of the kidney, functioning the same as a natural kidney and maintaining a similar structure (Morizane, 2015).

Cardiac organoids follow a similar process of differentiation to their kidney counterparts, but factors such as culture media, culture structure, and expressed transcription factors distinguish the two (Sahara, 2023). The development of cardiac organoids begins with the differentiation of human pluripotent stem cells into cardiac stem cells (Sahara, 2023). They are differentiated in a culture media typically containing BMP4, CHIR, FGF2, and FGF4 (Sahara, 2022). The addition of BMP4 and CHIR to the culture media is common to the development of both kidney and heart organoids, and they play an important role in stem cell differentiation and also prevent the degradation of beta-Catenin, which is necessary for cell adhesion especially in the heart (Kerr, 2023). FGF2, or fibroblast growth factor 2, is unique to the culture media of heart organoids (Itoh, 2016). This protein is crucial for cell growth, wound healing, angiogenesis, and embryonic development (Itoh, 2016). Additionally, FGF4 carries a similar function, and it facilitates the differentiation of HPSCs into atrium and ventricle chambers adjacent to those of in vivo embryonic hearts (Itoh, 2016). Cardiac organoids are almost exclusively grown in 3D cultures as they better resemble the structure and function of native hearts in comparison 2D cultures, which are unable to mimic the morphological and pathophysiological processes of in vivo hearts (Sahara, 2023). Additionally, 3D cardiac organoid cultures utilize biological scaffolds, which are typically made up of materials such as collagen, to promote and provide the framework for cell differentiation (Sahara, 2023). These differentiated cardiac stem cells form spherical aggregates that make up the inner layer of cardiospheres (Sahara, 2023). The outer layer of cardiospheres is made up of vascular smooth muscle cells and endothelial cells, enabling contractile function and some blood flow (Sano, 2022). Cardiac stem cells express the c-kit protein, a receptor tyrosine kinase thought to mark cardiac stem cell populations with the capability to repair heart tissue (Tenreiro, 2021). Although the expression of the exact transcription factors required for cardiac self-renewal have not been fully elucidated, it is expected that cardiac transcription factors such as Gata4 and Mef2c are required for cardiomyocyte induction due to their role in renewal and growth of cardiac cells (Navaee, 2023). Cardiac stem cells differentiate into epicardiac and myocardial cells, forming structures similar to that of a native heart (Mehanna, 2022). Heart organoids are able to maintain their vascularization following transplantation in mice and function to promote heart development as well as cardiomyocyte proliferation and maturation (Sano, 2023). Finally, this differentiation process results in the

development of a heart organoid, comprising cells analogous to the native heart with the possibility of greater similarity in function and structure for humans in the future (Sano, 2023).

Lastly, lung organoid development also primarily adheres to the aforementioned processes, yet the generation of this organoid still has a few key differences (Jacob, 2017). Lung organoids are first formed from the differentiation of human pluripotent stem cells into alveolar epithelial type 2 cells (AEC2s) (Jacob, 2017). AEC2s are crucial to lung development and function as they are alveolus progenitors and also synthesize surfactant, which is necessary to maintain open alveoli (Liu, 2024). HPSCs are differentiated in a culture media containing CHIR, BMP4, retinoic acid, and keratinocyte growth factor (Jacob, 2017). CHIR and BMP4 are utilized in the culture media of all three organoids (Kerr, 2023). Retinoic acid promotes AEC2 differentiation as this signaling enables the transition to mature lung tissue while keratinocyte growth factor prevents AEC2s from differentiating into AEC1s, which are thinner and more vulnerable to injury (Fernandes-Silva, 2020). Lung organoids, or alveolospheres, can be grown in both 2D and 3D cultures (Jacob, 2017). However, cultivating alveolospheres in 2D culture results in the differentiation of AEC2s into AEC1s, which lack self-renewing capability, whereas utilizing 3D culture prevents such differentiation and maintains self-renewing AEC2s (Liu, 2024). Alveolospheres express NKX2-1 and SFTPC (Katsura, 2020). NKX2-1 is an essential gene for lung cell differentiation as well as surfactant production while SFTPC is a gene that enables the production of surfactant protein C synthesized in AEC2s, lowering the surface tension within the lungs (Katsura, 2020). This differentiation process produces a lung organoid of the alveolus that maintains the same function and similar structural components of native alveoli.

Overall, the differentiation of organoids of the kidneys, heart, and lungs follow a similar process--undergoing stem cell differentiation into the respective specialized cell type, cultivated in a culture media with a specific selection of chemicals, and grown in the most suitable culture structure with respect to the desired formation of the organoid itself. Kidney organoids are the most similar to native structure and function due to its self-differentiating and greater renewal properties (Morizane, 2015). In contrast, heart organoids are currently the least analogous to their native counterparts in terms of structure and function due to the heart's limited renewal properties, complex structural components, and various functional necessities (Sahara, 2023). Like those of the kidney, lung organoids of the alveolus function almost identically to their native equivalent, however they also maintain the same spherical structure as organoids of the heart (Jacob, 2017).

Applications and Limitations

Organoids, having structure and function nearly analogous to their native organ counterparts, serve a variety of purposes in the field of regenerative medicine, disease modeling, and therapies (Yang, 2023). Since the function, structure, and therefore purpose of each organoid differs based on the respective conditions, they are applied in different ways (Yang, 2023). Furthermore, the application breadth of such organoids can only extend to the capabilities of the organoid itself, limited by differing levels of complexity, variations in development, as well as depth of research and discovery (Yang, 2023).

Kidney organoids are made up of self-organizing nephrons containing structures resembling in vivo kidneys (Morizane, 2015). In both 2D and 3D cultures, kidney organoids function with about 90% of the efficiency of native kidneys in vitro (Morizane, 2015). 3D kidney organoids are more desirable as they better represent the structure of in vivo kidneys (Morizane, 2015). They are utilized in studies for kidney development, pathophysiology, injury, drug testing and cell replacement therapies (Morizane, 2015). These organoids are the first to model human kidney development, and they serve as an important tool to better understand this vital organ (Nishinakamura, 2023). Common limitations for the clinical applications of kidney organoids include the variability of organoid culture, as they consist of different ratios of cell composition and culture media additives, leading to low and imprecise reproducibility (Nishinakamura, 2023). Additionally,

developmental protocols, especially regarding the duration of Wnt exposure greatly impacts the viability and specialization of nephron progenitors into either proximal or distal nephrons (Nishinakamura, 2023). The lack of immune cells also makes kidney organoids susceptible to disease and reduces in vivo viability (Nishinakamura, 2023). Finally, the limited vascularization of kidney organoids hinders its in vivo applications, imposing a risk of restricted blood flow and filtration (Nishinakamura, 2023). Although kidney organoids are not yet available for clinical transplantation, they are a powerful aid in advancing the field of regenerative medicine, with future prospects as a possible replacement for human-derived transplantation as current limitations are overcome (Nishinakamura, 2023).

Heart organoids comprise cardiac stem cells differentiated into specialized components of the native heart, resembling structures such as epicardiac and myocardial layers and maintaining some contractile and vascular functions in certain studies (Drakhlis, 2021). Cardiac organoids are rarely grown in 2D cultures as they are unable to replicate the morphology and pathophysiology of the native heart (Sahara, 2023). Due to this, heart organoids are mainly cultivated in 3D cultures utilizing biological scaffolds, enabling the formation of cardiac structures that better resemble their native counterparts (Sahara, 2023). Currently, heart organoids have been used as a tool to model cardiogenesis, disease, drug testing as well as screening (Mehanna, 2022). In addition, some cardiac organoid models are utilized for studies in regenerative medicine and transplantation (Tenreiro, 2021). However, the human heart is arguably one of the most complex and challenging organs to understand, resulting in an array of limitations that have impeded further application and development of these organoids (Mehanna, 2022). Present-day methods and protocols for cardiac organoid development remain unstandardized, resulting in variation as well as imprecision in reproducibility (Mehanna, 2022). This mirrors the issue of varying protocols for kidney organoid development, limiting the scope of production and applicability for both distinct organoid types (Nishinakamura, 2023). Furthermore, insufficient vascularization hinders the growth and function of cardiac organoids, impeding their utility as potential transplantable material (Mehanna, 2022). Lastly, current cardiac organoids lack both an immune system and a nervous system, preventing accurate replication of natural heart functions and communications (Mehanna, 2022). Despite these modern constraints, cardiac organoids remain pivotal for developmental, pathophysiological, and morphological research, and they possess the exciting ability to transform human health, particularly regarding transplantation, through refined research and developmental processes in the future.

Lung organoids, particularly those of the alveoli, are composed of specific alveolar cells that function the same as their native counterparts, producing surfactant and maintaining analogous structure (Jacob, 2017). The usage of 3D culture to grow these organoids is optimal, as it enables the organoid to self-renew and remain as the desired cell type (Jacob, 2017). Lung organoids are often applied to model lung development and disease, test drugs and certain therapies, and also to facilitate high-throughput screening for drug development (Parekh, 2020). These organoids serve as a novel approach to human lung regeneration, transplantation, and other clinical applications, but certain limitations continue to hinder that process (Parekh, 2020). Similar to both the development of heart and kidney organoids, the process of differentiating cells into lung organoids is not yet uniform, resulting in difficulty with replicating organoid models across multiple studies (Parekh, 2020). Additionally, translation of organoid models into their human applications is challenging as lung organoids grown in vitro often possess many morphological and physiological differences from their in vivo counterparts, limiting comparability (Jacob, 2017). Isolating specific lung progenitor cells is also a complicated process, causing a lack of autologous cells for transplant (Konda, 2020). Finally, the conversion of rodent models to clinical approaches regarding applications of lung organoids for human lung repair and function remains limited due to the physiological differences between the two organisms (Jacob, 2017). However, through greater research efforts and the overcoming of current limitations, lung organoids provide much hope for the future of transplantation and human health as a whole.

Ultimately, organoids harness the power to transform biomedical, physiological, and pathological studies (Yang, 2023). They serve as a new avenue for research, functioning as representative models that enhance

understanding of the human body (Yang, 2023). Organoids provide a fresh outlook onto the treatment of debilitating diseases and organ failure, with the potential for clinical applications that can change the lives of countless patients (Yang, 2023). The main obstacles that hinder the scope of current organoid applications are the existing limitations. Across the three organoids types studied, many of the same limitations persist. The most surmountable of these limitations is the lack of standardization of organoid development processes (Nishinakamura, 2023). Reproducibility and replicability of organoids widely differs among studies due to the absence of a systemized method. With greater collaboration among researchers, a uniform process for organoid differentiation can be developed, thus eliminating this limitation.

Conclusion

Across the United States, transplantations of the heart, lung, and kidneys are most often necessary to sustain and improve the lives of patients ("Organ Donation Statistics," 2025). However, limited access and availability of transplantable organs leaves many patients at risk of worsening conditions ("Organ Donation Statistics," 2025). Organoids serve as a potential solution to this issue (Yang, 2023).

Organoids are created through a specific process involving the differentiation of stem cells into small masses of cells with similar structure and function to their native organ equivalents (Yang, 2023). Due to its existence as a novel field in research, many uncertainties and limitations regarding organoid development persist, hindering the further implementation of organoids into clinical applications (Yang, 2023). The purpose of this paper is to compare the similarities and differences of the development as well as the specific limitations of each specific organoid in order to consolidate dispersed organoid research, ultimately highlighting approaches to better understand and overcome obstacles in this field.

Stem cells are the basis of organoid development, and all three organoid types utilize human pluripotent stem cells (HPSCs) (Zhu Z, Huangfu D, 2013). Greater access to HPSCs is essential for the increased production of organoids. Additionally, other stem cell types such as endogenous stem cells and cardiac stem cells are more particularly selected for use in the development of lung organoids and heart organoids, respectively (Mehanna, 2022). Differentiation is the process by which these organoids are actually created, following the same essential steps for each organoid type (Morizane, 2015). During organoid development, stem cells are first placed into a specific culture media including the chemicals BMP4 and CHIR (Kerr, 2023). In addition, the structure of the culture media also impacts the formation of the organoid itself, and it can be concluded that 3D cultures typically result in a more morphologically accurate organoid structure than their 2D counterparts (Morizane, 2023). Following differentiation, the newly developed organoid possesses similar structural and functional ability to the organ they mimic (Morizane, 2023). However, it can be seen that kidney organoids are most analogous in structure and function to in vivo kidneys whereas cardiac organoids prove to be the least similar in these aspects, reflecting the complexity and differences in understanding of each organ (Sahara, 2023). Currently, these organoids have mainly been used for disease modeling, the study of organ development, and drug testing (Yang, 2023). Some clinical applications have begun to be studied, but a multitude of limitations hinder the scope of such implementation. Limitations in organoid development are specific to the organoid type, but the most common limitation is the lack of a standardized differentiation process (Nishinakamura, 2023). This absence of uniform methodology is cited as a major limitation for the development of all organoid types, yet this issue can be overcome through collaborative effort among researchers to systemize this process.

By better understanding the intricacies of organoid development, researchers can advance the application of organoids to transform scientific knowledge and human health. With the potential to replace human-derived transplantation and improve function of heavily damaged organs, organoids are the future of medicine (Yang, 2023).

Since organoid development is an emerging field in biomedical science and regenerative medicine, many existing studies have yet to be fully conclusive in their results due to limited consensus among researchers regarding development processes (Yang, 2023). Current research on organoid development is extremely novel. Each study researched for this review was published within the last decade, citing a lack of prior studies and standardized methodology as the main reason for limited scope of research. It is expected that through further research and longer studies, available research and the extent of information will be much more encompassing in the future.

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Abstract

This review highlights the relationship between foods and the brain, specifically the hippocampus. This study aims to identify foods that have a positive correlation to cognition. Through reviewing research and experimental papers examining the nutritional effect on hippocampal functions, it is proven that iron, Vitamin C, and omega-3 fatty acids play a significant role in enhancing cognitive functions of the brain, especially in adolescence. These specific foods were chosen through papers detailing the positive impacts on cognition; whether it was increased memory, mental focus, attention span, etc. With this knowledge, incorporating brain foods into diet can reduce mental fatigue and memory loss and increase memory, focus, and cognition. This research matters because finding preventive methods for neurodegenerative diseases is equally as important to studying how to treat those already living with these diseases. Correlation Between Brain Foods and Cognition What is "brain food" and how do these foods improve cognition in the hippocampus? The purpose of this review is to explore the relationship between food, whether that be vitamins, minerals, or nutrients, and the hippocampus, a brain organ responsible for many cognitive functions including memory and learning. Due to the complexity of the human brain as individuals age, we've chosen to focus on the relationship between food and cognitive function during adolescence, and disregard exercise's effect on this relationship. It is possible that certain foods have the ability to improve cognition and can later help prevent neurological diseases down the line. Cognition is the mental action or process of acquiring knowledge and understanding through thought, experience, and the senses; it helps us to think, learn, remember, problem solve, and understand the world around us (National Cancer Institute). Impaired cognition is described as memory loss, trouble concentrating, completing tasks, understanding, remembering, following instructions, and solving problems (National Cancer Institute). In 2013, 5.3% of adults reported having cognitive trouble, this statistic increased to 7.4% in 2023 (BRFSS). Considering the significance of cognition in our everyday lives, this highlights the need to increase our cognitive capability; one of the ways being by focusing on "brain foods". Brain foods are described as foods that can boost brain power and improve memory, focus, and concentration (National University, 2018). More specifically, the part of the brain associated with these crucial cognitive functions like thinking, decision-making, and memory is the hippocampus. The hippocampus is located in the medial temporal lobe of the brain, on both sides, and has three major parts: the dentate gyrus, the hippocampus proper, and the subiculum. The dentate gyrus acts as the preprocessor of incoming information, preparing it for subsequent processing (Jonas and Lisman, 2014). The hippocampus proper is associated with memories, whether it's forming, storing, or organizing them. The subiculum collects information and sends it to other parts of the brain, aiding with memory retrieval.

According to Harvard Health, the brain has a direct effect on the stomach and what is eaten directly affects the structure and function of the brain. More information passes between the brain and gut than any other body system and there are more nerve cells in the gut than anywhere else in your body (Cleveland Clinic, 2023). Ingestion of foods leads to the release of hormones that reach centers in the brain like the hippocampus, which then promote synaptic activity, contributing to learning and memory (Gómez-Pinilla, 2015). As a result of the closely connected gut-brain axis, certain foods, like iron, zinc, and Vitamins B and C, have the capability to optimize cognitive function. This research is important because food has a serious impact on the development and cognition of the brain, therefore brain foods should be seen as essential to healthy and optimal capability. Therefore, we will specifically review research conducted on iron, Vitamin C, and omega-3 fatty acids and their effects on cognition through the hippocampus.

Cognition

Cognition is the mental action or process of acquiring knowledge and understanding through thought, experience, and the senses. It is essential to every aspect of human life, from knowledge to awareness, and optimal cognitive function is beneficial in many ways. For example, being able to problem-solve and learn new skills quickly are examples of optimized cognition. Moreover, cognition plays a huge role in education, especially at young ages. A study by Parisi et al. (2012) related greater educational attainment and participation in intellectual activities to better performance on cognitive measures. While this is one method, another method to increase cognitive function is by consuming brain foods, foods that boost brain power and improve memory, focus, and concentration. Through the gut-brain axis these foods directly influence the hippocampus, ultimately leading to a positive effect in cognitive processes. This axis includes the enteric nervous system- the neural network that works within the gastrointestinal tract to control its digestive functions. It has more than 500 million neurons making it the most complex neural system outside of your brain. The vagus nerve is the main linkage between the brain and the enteric nervous system. This nerve sends information about the conditions inside your gut from your enteric nervous system to your brain (Cleveland Clinic, 2023). By understanding this close relationship, it is evident that nutrition is crucial for the support and enhancement of healthy cognitive function.

Structure and function of the hippocampus

The hippocampus is involved in many functions including thinking, decision-making, and memory. It carries out these processes through its three main parts: the dentate gyrus, cornu ammonis and subiculum. Specifically, the dentate gyrus, the part of the hippocampus responsible for memory consolidation and spatial navigation, processes spatial memories and is involved in decision making. The hippocampus proper is responsible for memory formation, organization, and storage. Furthermore, the hippocampus works closely with other parts of the brain, like the amygdala, to process the memories created and stored in the hippocampus (Cleveland Clinic, 2024). Effectively, it helps the brain to plan and infer future events by combining and restructuring memories, a process crucial to flexible cognition. Flexible cognition, the ability to adapt your thinking and behavior in response to changing demands, is critical for shifting ideas, problem-solving, and overall adaptation to change. One brain food known to improve cognition is iron. A study conducted on school-age children found that iron supplementation had a significant positive effect on the intelligence, attention and concentration, and the memory of these children (Gutema et al., 2023). Foods high in iron are red meats, nuts, and leafy greens. The hippocampus supports the ability to bind and flexibly represent elements of an experience and permits the expression of flexible and adaptive behavior (Rubin et al., 2014). Vitamin C was found to increase work motivation and attentional focus, and contribute to better performance on cognitive tasks requiring sustained attention (Sim et al., 2021). Foods that are high in Vitamin C are citrus fruits. Both iron and Vitamin C work hand in hand because Vitamin C helps with the absorption of iron. Additionally, vitamin cobalamin plays an essential role in proper brain development and function (Melgar-Locatelli et al., 2023). These findings illustrate how together the hippocampus and cognitive-enhancing nutrients can promote flexible cognition and memory.

Memory

One of the most crucial functions of the brain is memory: the faculty by which the mind stores and remembers information. Memory can be impaired in many ways, for example, stress, lack of sleep, physical and mental illnesses, and certain neurodegenerative diseases. There are different types of memory: short-term, long-term, and working. Short-term memory is described as faculties of the human mind that can hold a limited amount of information in a very accessible state temporarily. This type of memory is useful for following a conversation or reading this sentence. Another form of memory is long-term memory: the vast storage of

knowledge and a record of prior events. This form of memory is useful for storing learned facts, skill, and information. The last form of memory is working memory which is used to plan and carry out behavior (Cowan, 2009). On this matter, one brain food found to improve memory is omega-3 fatty acids. In a study investigating whether or not eicosapentaenoic acid, an unsaturated omega-3 fatty acid, could benefit cognition, it was found to improve memory speed and accuracy (Patan et al., 2021). Mental fatigue is defined as a psychobiological state of tiredness caused by prolonged periods of performing demanding, cognitive-load-inducing activities, and it reduces efficiency in cognitive performance (Kunasegaran et al., 2023). Interestingly, creatine, a supplement used to enhance sports-fitness performance, was found to correlate positively with recognition memory and reduce mental fatigue (Rae et al., 2003). Additionally, neurogenesis, the birth and maturation of neurons in the dentate gyrus of the hippocampus, makes possible the long-term consolidation of new memories (Melgar-Locatelli et al., 2023). This process contributes to turning short-term memories into long-term memories, crucial for daily function, problem-solving, and acquiring skills. Polyphenols are known for their biological antioxidative, neuroprotective, and cognitive properties, considered as exogenous molecules able to modulate adult neurogenesis (Melgar-Locatelli et al., 2023). Specific nutrients can help strengthen memory, reduce mental fatigue, and support the brain's function of forming and storing memories.

Conclusion

Certain nutrients, vitamins, and minerals act as "brain foods" that enhance hippocampal function by supporting neurogenesis, synaptic activity, and memory formation, proving how proper nutrition during adolescence can significantly improve cognitive performance and help prevent future neurological decline. Additionally, parents can be aware of the best possible foods to support healthy and optimal brain development of their children. Lastly, teenagers and young adults can incorporate these brain foods into their diets to help them in their studies by improving memory, problem-solving skills, and attention span. Cognition, the mental process of knowing, is a skill of the brain that people across all stages of life use everyday. Cognition is vital for learning, problem-solving, and overall mental performance. Brain-boosting foods that affect the gut-brain axis, the communication network between the gastrointestinal tract and the central nervous system, and hippocampus can enhance cognitive function, such as memory, learning, and adaptation. The hippocampus plays a crucial role in learning and flexible cognition. Flexible cognition is described as the mental ability to switch between different concepts or ways of thinking and to think about multiple concepts at once. The hippocampus is also important to the process of memory; long-term, short-term, and working. Nutrients such as omega-3 fatty acids and creatine have been found to improve memory performance through enhancing memory speed as accuracy, respectfully. Additionally, nutrients like iron and vitamin C enhance processes like learning and adaptation by improving attention, memory, and neuron growth in the hippocampus, ultimately improving cognitive performance.

This review focuses on subjects under the age of 50 and takes into account solely diet's effect on cognition. More research should be done on adolescent's cognitive improvements through food to help prevent serious neurological diseases. This research matters because humans never stop learning, no matter how old they are. By finding ways to make attaining knowledge easier through eating memory-improving foods, many lives are helped. While research on neurodegenerative diseases is important, research on prevention and maintenance is equally as important. More research should be conducted on how to enhance cognition function through consuming brain foods earlier on in life before it gets to the point of a disease. Eating is essential to life, making the incorporation of brain foods in our diets simple and efficient. By taking daily supplements of iron, Vitamin C, and fish oils, cognition can be improved significantly.

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Central Roles of Mitochondrial Dysfunction and Oxidative Stress in Huntington's Disease: Mechanisms of Neurodegeneration and Emerging Therapeutic Strategies

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Abstract

This review examines the central roles of mitochondrial dysfunction and oxidative stress in the progression of Huntington's disease (HD), and explores therapeutic strategies that may help prevent or slow the progression of HD. Mutant huntingtin (mHTT) disrupts mitochondrial biogenesis, impairs mitophagy, and alters energy metabolism, which leads to excessive reactive oxygen species (ROS) production and neuronal death. These results demonstrate a continuous feedback loop between mitochondrial damage and ROS overproduction, which drives bioenergetic failure and accelerates neurodegeneration, particularly in medium spiny striatal neurons. Major findings within therapeutic strategies include targeting these interconnected mechanisms through mHTT lowering compounds, iron chelation, metabolic rescue, and mitochondrial stabilization. Evidence also supports approaches that promote mitophagy and restore transcriptional regulation to improve mitochondrial health. Overall, evidence suggests that multi-targeted approaches are needed to intercept the ROS- mitochondrial cycle and improve outcomes for individuals affected by HD. Continued investigation into these pathways will be essential for developing effective, clinically translatable treatments.

Introduction

Huntington's disease (HD) is a progressive, inherited neurodegenerative disorder that profoundly impairs motor, cognitive, and psychiatric function. Symptoms typically include involuntary movement (chorea), impaired coordination, cognitive decline, mood and attention disturbances, and difficulty speaking and swallowing. These functional impairments worsen over time, eventually leading to severe disability, loss of independence, and a lessened quality-of-life. Globally, HD affects 5 individuals per 100,000, with approximately 30,000 people currently living with a diagnosis in the United States (Rawlins et al., 2016). Each year, thousands of new diagnoses are projected to be made, yet there is currently no curative treatment available.

At the cellular level, the pathology of HD is most pronounced in the striatum of the basal ganglia, a brain region critical for motor control and motivation, and reward processing. The striatum is composed primarily of medium spiny neurons (MSNs), which rely on excitatory glutaminergic inputs from the cortex and dopaminergic inputs (DA) from the substantia nigra (SN) and ventral tegmental area (VTA). In HD, MSNs are disproportionately vulnerable to degeneration, disrupting normal communication with striatum and midbrain dopaminergic (DA) neurons (Raymond et al., 2011). This selective vulnerability contributes to the hallmark motor dysfunction and psychiatric features of the disease. While the genetic cause of HD, an expanded CAG repeat in the HTT gene, is well established, it is still crucial that researchers continue to investigate the molecular mechanisms that accelerate neuronal death.

This paper focuses on two interconnected processes that play a central role in HD pathogenesis: mitochondrial dysfunction and oxidative stress. Mitochondria are essential for energy production (ATP), calcium regulation, and cellular homeostasis which promotes survival. In HD, mutations in the huntingtin protein (mHTT) impairs normal mitochondrial function by impairing mitochondrial transport along axons, reducing respiratory chain activity, and disrupting calcium buffering. These deficits are especially damaging in

striatal MSNs, which already have high metabolic demands due to their dense synaptic inputs. As mitochondrial efficiency declines, these neurons face chronic energy deficits, destabilized calcium homeostasis, and increased susceptibility to apoptosis. Closely tied to these changes is oxidative stress, a state in which reactive oxygen species (ROS) overwhelm the cell's mitochondria and antioxidant defenses. ROS are generated naturally as byproducts of mitochondrial oxidative phosphorylation, but in HD, impaired mitochondrial respiration leads to excessive ROS production. Elevated ROS levels cause widespread cellular damage, including contributions to protein misfolding, lipid peroxidation, and damage to mitochondrial DNA (Johri & Beal, 2012). In the striatum, this oxidative burden accelerates MSN degeneration, creating a vicious cycle in which mitochondrial dysfunction and oxidative stress reinforce each other to drive progressive neurodegeneration and neuronal death.

Given the lack of curative options, it is incredibly important that novel therapeutic strategies increasingly aim to target underlying mechanisms of accelerated neurodegeneration within HD. Approaches include antioxidants to reduce ROS accumulation, compounds that stabilize mitochondrial function, and experimental gene therapies designed to correct downstream effects of the HTT mutation (Franco-Iborra et al., 2021). Although none of these strategies have yet achieved clinical success, they demonstrate the urgent need for continued research into the relationship between mitochondria and oxidative stress in HD pathology.

This paper seeks to address the roles of mitochondrial dysfunction and their interaction with oxidative stress to accelerate neurodegeneration in Huntington's disease (HD). Additionally, this paper seeks to highlight some potential therapeutic strategies that may serve to target these processes. Understanding this relationship is critical not only for clarifying disease mechanisms that contribute to neurodegeneration, but also for developing interventions that may slow disease progression and improve quality of life for individuals living with HD.

Mutant Huntingtin and Mechanisms of Mitochondrial Dysfunction in HD

In healthy neurons, mitochondria are essential for maintaining cellular energy balance and neuronal survival. They are known to create ATP (energy) for the cell through the process of cellular respiration, buffer cytosolic calcium, and regulate apoptotic signaling. Mitochondria constantly undergo fission and fusion cycles to maintain their integrity, and dysfunctional mitochondria are selectively degraded through mitophagy, ensuring a healthy mitochondrial network to efficient synaptic transmission.

In Huntington's mitochondria, the mutant huntingtin (mHtt) causes disruption of mitochondrial function. One mechanism by which mHtt disrupts mitochondrial function involves transcriptional repression (by stopping the expression) of PGC-1alphaa transcriptional coactivator that regulates genes responsible for mitochondrial biogenesis, oxidative metabolism, and antioxidant defense (Cui et al., 2006).

In a study by Cui et al., 2006, cellular models were used to express the wild type of hunting (Htt) to monitor ROS and mitochondrial health and function. The results demonstrated that mHTT binds to transcription regulators and suppresses PGC-1alpha expression, resulting in decreased mitochondrial biogenesis and reduced respiration. Additionally, themHtt in this experiment was concluded to target the polyQ expansions present on PGC-1alpha, which impairs mitophagy by disrupting defective mitochondrial clearance and accumulates dysfunctional mitochondria and ROS (Franco-Iborra et al., 2021). As a result, reduced PGC-1alpha signaling diminishes mitochondrial biogenesis, leading to compromised energy availability and rendering neurons more susceptible to excitotoxicity, oxidative stress and synaptic instability.

Another critical factor in mitochondrial dysfunction is the disruption of intracellular signaling pathways that regulate energy metabolism and stress responses. One significant pathway involved is the mechanistic target of rapamycin complex 1 (mTORC1), a nutrient and energy sensing kinase that regulates metabolic cues to balance anabolic and catabolic processes. Under normal conditions, mTORC1 activity promotes cell growth when energy and nutrient levels are sufficient, while its inhibition activates autophagy during nutrient scarcity.

In HD mHtt hyperactivates mTORC1 signaling, resulting in metabolic dysregulation, impaired autophagy, and increased cellular stress. Pryor et al., 2014, demonstrated that mHTT interacts with Rheb, an upstream activator of mTORC1, thereby enhancing its signaling and shifting neuronal metabolism towards growth and protein synthesis even under stress conditions. This maladaptive activation prevents cells from initiating autophagy, a critical mechanism for clearing misfolded proteins and damaged organelles. Consequently, neurons accumulate defective mitochondria and toxic aggregates of mHTT, amplifying cellular stress. These findings demonstrate that HD disrupts mitochondrial function and impairs energy signaling and clearance of defective organelles, which compromises neuronal survival and accelerates degeneration of striatal MSNs by creating a cellular environment prone to oxidative stress.

Mitochondrial regulation depends on mitophagy, a specialized form of autophagy responsible for the selective degradation of damaged mitochondria. Under physiological conditions, damaged mitochondria trigger the recruitment of PTEN-induced kinase and the E3 ubiquitin ligase Parkin, which label dysfunctional mitochondria for degradation. In HD, this process is severely impaired. Franco-Iborra et al., 2021, found that mHTT interferes with the recruitment of mitophagy receptors, such as optineurin, preventing efficient clearance of defective mitochondria. As a result, dysfunctional mitochondria accumulate within neurons, producing ROS. This accumulation not only increases oxidative stress but also perpetuates a feedback loop in which damaged mitochondria produce more ROS, further damaging mitochondrial DNA and leading to fragmented MSNs vulnerable to degeneration.

In addition, HD neurons exhibit impairments in cellular energy metabolism. Studies using induced pluripotent stem cell (iPSC)-derived neurons from HD patients have provided valuable insight into metabolic deficits. The HD iPSC Consortium (2020) demonstrated that HD-derived neurons showed reduced glycolytic capacity, decreased oxidative phosphorylation, and lower intracellular ATP concentrations compared to control neurons. These findings suggest that both cytosolic and mitochondrial energy pathways are compromised. Because striatal MSNs depend heavily on oxidative metabolism to maintain homeostasis in excitatory-inhibitory signaling, even modest ATP reductions can severely disrupt neuronal firing and synaptic plasticity. Moreover, impaired glycolysis in glial cells further limits the supply of lactate, an alternative energy substrate for neurons, compounding the bioenergetic deficit (Polyzos et a., 2016). This global energy failure contributes to synaptic dysfunction, reduced NT synthesis, and ultimately neuronal death.

Collectively, these findings reveal that mHTT disrupts mitochondrial function through converging mechanisms, repression of biogenesis, dysregulation of metabolic signaling, failure of mitophagy, and systemic energy depletion. The combination of these defects undermines neuronal survival and creates an intracellular environment prone to oxidative stress, energy dysregulation,and apoptosis. Understanding these pathways provide important insight into why striatal neurons are especially vulnerable in HD and highlights potential targets for therapeutic intervention.

Oxidative Stress and its Interaction with Mitochondrial Dysfunction in HD

In a healthy neuron, oxidative stress is managed through a balanced system of enzymatic and non-enzymatic antioxidants, quality control mechanisms for mitochondria, and activating DNA repair pathways. Neurons generate reactive oxygen species (ROS) as natural byproducts of oxidative phosphorylation in the mitochondria, but they are normally neutralized by enzymatic antioxidants such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, along with non-enzymatic antioxidants like vitamin E and coenzyme Q10 (Sie & Jones, 2020). In order to produce antioxidants, the Nrf2-ARE (antioxidant response element) pathway crucially activates the transcription of genes encoding detoxifying and antioxidant enzymes. These mechanisms collectively maintain redox homeostasis and protect mitochondria DNA (mtDNA), proteins, and membranes from oxidative damage. Mitochondrial quality control pathways, including mitophagy, ensure that oxidatively damaged mitochondria are removed, while DNA repair systems such as

base excision correct oxidative lesions that arise in mtDNA.

In Huntington's disease, (HD), mitochondrial quality control collapses. Mutant Huntington (mHTT) expression leads to excessive amounts of oxidative stress, which produces an excess of reactive oxygen species (ROS) that overwhelm the cell's antioxidant defenses and damages mtDNA. Studies have shown that HD neurons exhibit increased levels of superoxide anion, hydrogen peroxide, and hydroxyl radicals, which inflict oxidative damage on mtDNA and compromise mitochondrial bioenergetics (Siddiqui et al., 2012). Additionally, mHTT impairs the Nrf2-ARE signaling pathway by disrupting nuclear translocation and preventing upregulation of these protective genes. MtDNA is particularly vulnerable because it lies in close proximity to the electron transport chain where ROS are generated and lacks the protective histones found in nuclear DNA. As a result, base modifications, strandbreaks, and deletions impair the transcription of respiratory chain subunits, thereby reducing ATP synthesis and further disrupting energy metabolism. Furthermore, oxidative damage to mitochondrial DNA, proteins, and membranes compromises calcium buffering, membrane potential, and promotes neuronal apoptosis (Quintanilla et al., 2017). As these molecular

injuries accumulate, the neuron's capacity to maintain redox balance and energy homeostasis diminishes, allowing other amplifying factors of oxidative stress to further accelerate ROS generation and cellular damage. Another contributor to oxidative pathology in HD is iron accumulation, which serves as a potent amplifier of ROS generation.

Post-mortem analyses of HD brains reveal elevated levels of mitochondrial iron, particularly in the striatum, where iron participates in Fenton reactions that convert hydrogen peroxide into highly reactive hydroxyl radicals (Agrawal et al., 2018). The same study by Agrawal et al., 2018 demonstrated that excess mitochondrial iron leads to increased lipid peroxidation and oxidative injury which damages mitochondrial membranes and exacerbates bioenergetic failure. This iron-induced oxidative stress also disrupts ATP generation and accelerates the degeneration of MSNs which are already metabolically fragile. The build-up of iron creates a vicious cycle in which oxidative stress perpetuates mitochondrial dysfunction and amplifies neuronal vulnerability.

A bidirectional feedback loop exists between mitochondrial dysfunction and oxidative stress in HD. Impaired mitophagy prevents the clearance of defective mitochondria, allowing them to accumulate and generate additional ROS (Franco-Iborra et al., 2021). The resulting oxidative burden further damages healthy mtDNA, respiratory enzymes, and mitochondrial membranes, which in turn worsen mitochondrial performance and increases the number of defective organelles (Siddiqui et al., 2012). This positive feedback mechanism creates a self-perpetuating cycle where dysfunctional mitochondria produce excess ROS, and ROS further damage mitochondria. Over time, this loop leads to a bioenergetic crisis, calcium dysregulation, and neuronal death.

In summary, oxidative stress and ROS are both a product and a perpetuator of mitochondrial dysfunction in HD. Once oxidative damage begins, it forms a self-reinforcing cycle of mitochondrial failure and ROS overproduction that accelerates neuronal death. This feedback loop explains why neurodegeneration in HD becomes increasingly aggressive as the disease progresses. Consequently, it becomes even more important to develop therapeutic strategies that target oxidative stress, enhance mitochondrial efficacy, and restore antioxidant signaling in order to interrupt this destructive cycle and preserve neuronal function.

Emerging Therapeutic Strategies

Throughout the chaos of HD, researchers have worked to develop therapeutic strategies that can suppress symptoms and hopefully lead to a cure. Because there is still no clinical treatment that halts disease progression, emerging therapies increasingly aim to target the molecular pathways underlying oxidative stress, mitochondrial dysfunction, and mutant huntington (mHTT) toxicity.

One promising approach involves the use of small molecule inhibitors which directly reduce levels of mHTT. Bahat et al., 2024 explored this strategy using small molecule inhibitors of the transcriptional elongation factor, Spt5-Pol II, which regulates RNA polymerase II processivity and influences mHTT mRNA expression. The study utilized B6 HD mouse models carrying the human mHTT gene with expanded CAG repeats, which mimics the disease's progression in humans. The mice received direct intracerebral injections and oral administration of the inhibitors SP1-24 and SPI-77 to evaluate the long-term effect on disease pathology and behavior. Behavioral tests and molecular readouts were measured. Behavioral testing included a beam walk assay to measure motor coordination, anxiety-like behavioral testing, and cage return memory assessments. Results showed that treated mice exhibited lower mHTT levels in the brain, restored molecular markers, improved

motor coordination and reduced anxiety-related behaviors without observable side effects. By targeting Spt5, the inhibitors effectively suppressed transcriptional elongation of the mutant allele, thereby reducing the production of toxic mHTT protein and alleviating its downstream cellular effects.

Another emerging therapeutic strategy is iron chelation therapy, which reduces mitochondrial iron accumulation. Excess mitochondrial iron is a major driver of oxidative stress and neurodegeneration in HD, leading to cellular death. Mitochondrial iron overload also contributes to the formation of ROS, lipid peroxidation, mitochondrial membrane damage, and decreased ATP production. Agrawal et al., 2018 tested whether iron chelation could mitigate these effects by using Deferiprone (DFP), an iron-chelating drug, to inhibit the production of iron. In this experiment, he used two mouse models, one was a fast progressing transgenic model with severe HD pathology symptoms, while the other was a full-length, humanized model that mirrored the slower, chronic course of human HD. Agrawal dissected cortical tissue cortex from HD and control mice, isolated the mitochondria, and chemically measured their iron content. Results showed that the HD mice were found to have excessive amounts of iron in their cortical mitochondria. Additionally, mice treated with DFP were found to have lower levels of mitochondrial iron, restored ATP production, reduced oxidative damage, and improved motor performance. These findings suggest that iron chelation not only relieves oxidative stress, but also restores mitochondrial bioenergetics.

In addition to iron chelation and mHTT suppression, researchers are investigating neuroprotective factors that target mitochondrial function directly. Once such example involves the Engrailed proteins 1 and 2 (EN1/EN2), which are homeodomain-containing transcription factors essential during embryonic brain development and play critical roles in the survival, maintenance, and preservation of midbrain dopaminergic (DA) MSNs. As previously discussed, MSNs are the neuronal population most affected in HD. EN1 and EN2 regulate the expression of genes involved in mitochondrial energy metabolism and protect MSNs against oxidative and metabolic stress by enhancing the translation of complex I subunits in the mitochondrial respiratory chain. Alvarez-Fischer et al., 2011, demonstrated that these proteins also stabilize mitochondrial bioenergetics under stress conditions and protect neurons against complex I inhibition. By maintaining mitochondrial integrity, EN1/EN2 may help sustain neuronal survival in oxidative and metabolic stress environments similar to those observed in HD conditions.

Finally, metabolic rescue through glycolytic enhancement offers another avenue of intervention. Supplementation with glycolytic intermediates has been shown to improve ATP production and mitochondrial respiration in in-vitro models. The HD iPSC Consortium, 2020, reported that specifically, supplementing induced pluripotent stem cell (iPSC)-derived neurons from HD patients with metabolic substrates such as pyruvate or lactate restored bioenergetic balance and increased cell viability. iPSC-derived neurons are laboratory-grown neurons reprogrammed from adult human cells that are genetically modified to carry the same mutations found in patients. These cells mimic the molecular and physiological properties of human neurons affected by HD, allowing researchers to study disease progression and test therapeutic interventions in a controlled environment. Through these models, researchers observed that adding glycolytic metabolites enhanced ATP generation and reduced oxidative stress, compensating for the impaired energy metabolism

typical of HD neurons. This approach helps neurons meet their high energy demands despite mitochondrial dysfunction, highlighting a promising direction for future metabolic therapies.

Together, these findings highlight that therapeutic strategies targeting mHTT expression, iron accumulation, mitochondrial stabilization, and energy metabolism each address interconnected aspects of HD pathology. By intervening early in these pathways, and before oxidative stress and mitochondrial dysfunction form a self-reinforcing cycle, researchers are hopeful that these therapies will be effective in slowing disease progression and preserving neuronal function in patients living with Huntington's disease.

Discussion

Huntington's disease pathogenesis involves a continuous and self-perpetuating cycle between mitochondrial dysfunction and reactive oxygen species (ROS) generation. Mutant huntingtin (mHTT) disrupts mitochondrial biogenesis and impairs mitophagy, leading to an accumulation of defective mitochondria. These damaged mitochondria generate excessive ROS, which in turn damages mitochondrial DNA and impairs mitochondrial function. This vicious cycle exacerbates cellular energy deficits and contributes to progressive neuronal degeneration. Therefore, understanding the intricate relationship between mitochondrial function and ROS is critical for identifying effective therapeutic targets in Huntington's disease (HD).

Given the strong connections between mitochondrial dysfunction and ROS production, single target therapies are often insufficient. Effective treatment of HD will likely require multi-targeted approaches that can disrupt different points in the ROS-mitochondrial cycle. For instance, iron chelation has shown promise in reducing ROS by limiting mitochondrial iron accumulation, restored ATP production, reduced oxidative damage, and improved motor performance. These results are significant because excessive iron has been linked to disrupted transcriptional activity through Spt5-Pol II dysregulation. In HD models, restoring proper Spt5-Poll II function has been shown to normalize the expression of genes involved in mitochondrial maintenance, suggesting that combining iron chelation with transcriptional regulation may effectively restore mitochondrial redox balance.

Similarly, metabolic rescue strategies also demonstrate a multi-targeted approach by enhancing glycolysis and ATP production to restore cellular energy balance. Evidence from iPSC-derived neuronal models supports this, as HD neurons that have not had function restored by addition of glycolytic metabolites display reduced ATP synthesis and increased ROS even before cell death occurs. Therapies that restore metabolic efficiency and biogenesis in these models have improved neuronal survival, reinforcing the idea that early mitochondrial intervention can disrupt the disease cycle. Additionally, the transcription factor Engrailed has been shown to upregulate genes involved in the mitochondrial biogenesis and oxidative defense. Its neuroprotective role suggests combining metabolic or iron-targeting therapies with Engrailed modulation could strengthen mitochondrial function and overall cellular resilience.

Although both methods have demonstrated promising results in pre-clinical cell and animal models, translation to clinical trials remains challenging, likely because differences in dosing, safety profiles, and disease stage progression can influence clinical outcomes. Moreover, the lack of reliable biomarkers to monitor mitochondrial health and ROS levels in patients makes it difficult to determine optimal dosing to evaluate treatment efficacy and satisfy necessary benefits to mitochondrial health. However, collectively, the evidence suggests that mutli-targeted interventions, linking iron regulation, metabolic enhancement, and transcriptional control, may offer the most comprehensive benefit for mitigating neurodegeneration and improving mitochondrial health in HD.

Despite these preclinical successes, several limitations must be considered. Many of the studies reviewed were conducted five to seven years ago, meaning that newer discoveries or improved therapeutic methods may not have been incorporated into their findings. Additionally, variability among study populations, such as differences in disease duration and progression, can affect the reproducibility and generalizability of results.

While iron chelation, metabolic rescue, and transcriptional modulation therapies continue to show potential, they still lack large-scale clinical validation and consistent human data.

Looking forward, future research should focus on understanding mechanisms of mitophagy and mitochondrial DNA repair, as well as developing combination therapies that simultaneously target mitochondrial dysfunction, transcriptional regulation, and ROS imbalance. The identification of reliable biomarkers capable of tracking mitochondrial and oxidative changes in patients would greatly improve clinical trial design and therapeutic monitoring. Expanding on findings and revisiting metabolic-promoting effects of iPSC-derived neurons and Engrailed studies could help translate these laboratory discoveries into effective and safe treatments for HD.

Finally, there is a clear need for renewed investigation into these mechanisms. Many foundational studies were conducted years ago, and since then, advancements in genetic editing, high-resolution imaging, and stem cell technology could provide new insight into the mitochondrial-ROS connection to HD. Encouraging new research that integrates these modern methods will be critical for validating older findings, refining therapeutic strategies, and ultimately improving clinical outcomes for HD patients. The field must continue to evolve, moving beyond replication of early models towards innovative, translational studies that can bridge the gap between laboratory research and patient care.

In summary, the complex relationship between mitochondrial dysfunction and oxidative stress lies at the core of Huntington's disease pathogenesis. The evidence from studies on iron chelation, metabolic rescue, Spt5-Pol II, Engrailed, and iPSC-derived neurons collectively highlights how each mechanism contributes to both disease progression and potential recovery. Together, they reveal that no single approach can fully address HD's multifaceted pathology and that instead, integrated, multi-targeted interventions are required. By combining metabolic, genetic, and molecular strategies, and by investing in updated, interdisciplinary research, future studies can move closer to uncovering treatments that restore mitochondrial balance and improve disease outcomes and quality of life for those affected by Huntington's disease.

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Integrating Artificial Intelligence (AI) and Genetically Modified (GMs) Crops to Advance Ecological and Climate Resilience in U.S. Midwestern Agriculture

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Abstract

This paper examines how the integration of artificial intelligence (AI) and genetically modified (GM) crops can transform modern agriculture into a more sustainable and resilient system. With a focus on the United States Midwest, this study explores how GM technology restores ecological stability by reducing chemical fertilizer dependency, enhancing soil health, and supporting biodiversity. Using a literature-based analysis approach, the paper synthesizes research on AI-driven genetic modeling, predicted ecological feedback, and optimization of crop performance. Literature findings indicate that AI can identify gene combinations improving drought tolerance and nutrient efficiency, while GM crops reduce chemical fertilizer use and promote biodiversity. My findings indicate that the intersection of AI and GM technology enhances yield stability and fosters adaptive responses to shifting climate conditions, creating a framework to help mitigate greenhouse gas emissions and strengthen agricultural sustainability. The paper concludes that AI-assisted biotechnology offers a transformative pathway toward crop farming, aligning technological progress with long-term environmental and climate resilience goals.

Introduction

Agriculture in the United States (U.S.) Midwest is at the center of American food production, but it is increasingly vulnerable to various pressures from climate change. Rising temperatures, prolonged droughts, increased rainfall, and frequent tornado seasons have disrupted traditional growing cycles, placing strain on the growth of vital crops such as corn and soybeans (Merem, 2023). These changes threaten regional productivity and destabilize broader ecological systems, as the heavy reliance on monoculture and chemical inputs exacerbates soil degradation, pollinator decline, and greenhouse gas emissions (Ngongolo & Mmbando, 2021). Due to this, genetically modified organisms (GMOs) have become increasingly adapted to assist these systems. However, conventional methods of GMO development are time-intensive and limited in their ability to predict how crops will perform in rapidly shifting climates.

Advances in artificial intelligence (AI) provide a beneficial opportunity to transform this process. Machine learning algorithms can accelerate trait selection, model the long-term viability of new crop varieties under projected climate scenarios, and optimize seed design as a way to balance productivity with sustainability (Mmbando, 2022). Integrating AI into biotechnology can help agricultural science move toward more proactive systems that enhance ecological stability and mitigate the agricultural sector's contributions to climate change. GMO technology has already demonstrated major potential to stabilize yields and reduce environmental pressures under increasing climate variability (Merem, 2021). The intersection of genetic engineering and computational modeling is a crucial step in building resilience for one of the world's most productive agricultural regions. At stake is not only the future of Midwestern farming but also the capacity of the global food system to withstand intensifying environmental disruption.

Ecological Resilience and Sustainable Production in Midwestern Agriculture

The ecological stability of the U.S. Midwest is increasingly threatened by unsustainable agricultural practices and the effects of climate change. Decades of corn and soybean monoculture production have led to depleted soil nutrients and more disease outbreaks amongst insects because of less biodiversity. In response to

these difficulties, GM crops have emerged as a strategic intervention that is capable of restoring the balance within these strained ecosystems. GM crops reduce the excessive use of pesticides and fertilizers, thus lowering chemical runoff and enhancing the ecological balance of farming ecosystems (Ngongolo & Mmbando, 2021). This shift away from pesticide usage is critical in mitigating chemical overuse - one of the region's most persistent ecological threats. The excess nitrogen and phosphorus from fertilizers are primary contributors to soil degradation and eutrophication in nearby waterways, all leading to loss of biodiversity and long-term environmental harm. Genetically engineered crops with built-in pest resistance and nutrient efficiency could minimize these external issues and sustain or even increase yields.

GM crops also play a crucial role in promoting biodiversity and resilience against environmental shifts. A study found that "Midwestern farmers using GM seed varieties experience improved resistance to crop diseases, contributing to yield stability under environmental stress" (Merem, 2021). The disease-resistant varieties decrease the risk of widespread crop failure while reducing the need for monocultural planting strategies that threaten ecological diversity. In turn, healthier soils and diversified cropping systems promote beneficial insect populations essential for ecological balance. The ability of GM crops to maintain productivity without exhausting the surrounding ecosystem directly enhances long-term sustainability, even as extreme weather events become more frequent.

In addition to making crops more resilient against environmental stressors, the ecological stability of GM crops also contributes to the preservation of soil health and midwestern biodiversity. Deep root systems and efficient nutrient uptake of modified plants reduce erosion and nutrient leaching. These issues are two of the most critical causes of soil degradation across the midwestern farmlands. Healthier soils improve water retention and reduce flooding risks while contributing to carbon sequestration. Agricultural soil can be used as a partial offset to greenhouse gas emissions from other sources. Moreover, minimizing the need to tillage and replanting assist GM crop systems in sustaining the soil microbial communities essential for long-term soil fertility. These cumulative effects "support a regenerative cycle in which productivity, biodiversity, and environmental balance reinforce one another" (Ngongolo & Mmbando, 2021). The integration of genetically modified crops is not just an adaptation to current climate pressures; it represents a shift towards agricultural models that value ecological stability as well as yield. The midwest contains vast croplands and plays a historic role in US food production. The addition of GM crop technology stands at the forefront of this transition, where ecological stability and agricultural innovation can coexist as crucial goals for success.

Integrating Artificial Intelligence in Genetic Modification and Predictive Modeling

AI introduces a transformation to the ecological and agricultural benefits of genetic modification. AI modeling helps scientists evaluate ecological interactions precisely, ensuring that new GM crops strengthen rather than destabilize surrounding ecosystems (Ngongolo & Mmbando, 2021). Machine learning (ML) helps researchers simulate interactions between modified genes, soil conditions, microbial communities, and climate change variables before implementation into the agricultural world. The advanced capabilities of ML reduces the unintended ecological consequences that older GM technologically occasionally produced. For example, AI could help identify gene combinations that improve drought tolerance or nitrogen uptake efficiency, reducing the need for synthetic fertilizers that disrupt soil ecosystems. By integrating AI into biotechnology, the development of GM crops becomes faster and also more ecologically informed. It is guided by complex data on soil health, carbon sequestration potential, and biodiversity maintenance.

The application of AI in agricultural biotechnology is one of the most transformative advancements in modern crop science, in addition to ecological modeling. The traditional methods of creating GM crops relied on trial-and-error breeding, labor-intensive data collection, and long years of field testing to ensure performance and safety. Despite this, AI-driven computational models can analyze large genomic datasets and simulate many environmental variables. This assists scientists in predicting how genetic modifications will

perform long before they are physically implemented. According to a study, machine learning models can predict gene-trait associations with accuracy, substantially reducing the time and cost of crop improvement programs" (Chen et al., 2024). These models allow researchers to prioritize genetic combinations that optimize drought resistance, nutrient efficiency, and yield stability under specific climatic scenarios - an especially critical advancement for the U.S. Midwest, where environmental unpredictability is increasing with no bounds.

AI systems are also transforming how scientists interpret ecological and agronomic feedback. Algorithms that are trained on satellite imagery, soil composition data, and climate projections can adjust crop management strategies, highlighting that genetic design is an adaptive process rather than a static one. It has been found that AI-guided phenotype modeling integrates genomic and environmental data streams to dynamically refine breeding objectives in real time, accelerating the development of new GMO varieties and strengthening predictive accuracy regarding how crops interact with surrounding ecosystems (Xu et al., 2022). For example, models can forecast ways a new corn hybrid might influence nitrogen cycling or pollinator populations across multiple growing seasons.

AI-driven modeling tools are beginning to support farmers directly through decision-support systems as well. They can recommend optimal planting schedules, fertilizer levels, and hybrid selections based on localized climate and soil data. These tools, when integrated with GM crop varieties, create a feedback loop between digital prediction and biological innovation, allowing agriculture to adapt precisely to regional conditions. However, a study cautions that AI models risk reproducing data biases or overfitting predictions when trained on incomplete or regionally skewed datasets (Xu et al, 2022). To maintain accuracy and public trust, it is imperative to ensure that predictive systems are transparent, inclusive, and scientifically robust. Ultimately, the integration of AI in GMO development redefines what agricultural adaptation means - it transforms it from a reactive process into a proactive, continuously evolving system that aligns innovation with long-term sustainability.

Climate Mitigation through Genetic Innovation

Climate change poses an extreme threat to midwestern agriculture. Rising temperatures, shifting precipitation patterns, and increased tornado frequency are undermining traditional farming systems (Merem, 2021). The agricultural sector suffers from and contributes to these environmental disruptions through greenhouse gas emissions from fertilizer use, soil degradation, and energy-intensive production cycles. GM crops offer a path toward mitigation by addressing the factors that increase emissions and ecological destability. GM crops "reduce the excessive use of pesticides and fertilizers, thus lowering chemical runoff and enhancing the ecological balance of farming ecosystems" (Ngongolo & Mmbando, 2021). This is a process that curtails nitrous oxide emissions, which are potent greenhouse gasses produced by fertilizer overuse. Crops that are engineered for nutrient efficiency or drought tolerance require fewer inputs. This conserves both resources and energy, while maintaining productivity under increasingly threatening conditions.

GMOs also contribute to climate resilience by improving carbon sequestration and preserving soil health. They promote root structures that retain organic matter and prevent erosion. Additionally, certain genetically engineered crops support the storage of atmospheric carbon in the soil which helps offset agricultural emissions, and this is supported by AI-driven modeling that enables scientists to evaluate ecological interactions more precisely (Mmbando, 2024). This allows for the development of GM varieties that are optimized for carbon efficiency, nutrient cycling, and adaptation to future climate scenarios. The intersection of biotechnology and computational analysis represents an important advance in sustainable land management, where genetic modification serves as a productivity tool and also as a climate solution.

The long-term implications of the integration of AI and GM research extend past immediate emission reduction. Agriculture can stabilize yields and reduce the pressure to expand farmland by creating crops that can thrive in various different conditions, which helps especially when it is one of the major motivations for

deforestation. As the midwest continues to face erratic weather patterns, the use of genetically modified crops and AI-based environmental modeling could assist in a regional transformation toward regenerative agriculture. This model predicts the creation of farmlands that are both productive and carbon-conscious, where technology adapts to climate change and actively mitigates its effects. Essentially, the fusion of AI and genetic innovation positions agriculture not as a victim of the climate crisis, but as a critical agent in reversing it.

Conclusion

Integrating artificial intelligence with genetically modified crops is a crucial step towards a more sustainable and ecologically resilient agricultural system. AI-driven modeling enhances ecological stability by allowing scientists to predict and optimize gene-environment interactions with extreme precision. When applied to GM technology, the models ensure that innovation is guided by data-informed ecological information, not by trial and error. In the U.S. Midwest, agricultural productivity and environmental harm coexist, indicating the importance of these agricultural advancements. The ability to engineer crops that conserve soil health, biodiversity, and reduce synthetic fertilizer dependence will address the environmental challenges already posed by greenhouse gas emissions and leaching.

The role of AI-assisted genetic modification in mitigating climate change is equally as important. These technologies transform agriculture into an ally against climate change by improving drought tolerance and reducing greenhouse gas emissions. The future of sustainable farming depends on the balance of innovation and environmental sustainability. AI-guided GMOs have this balance, helping to shift from reactive to proactive forms of crop management.

The combination of artificial intelligence and genetic engineering is not only a technological milestone, but also a philosophical one. It reflects the growing capacity of humanity to harmonize innovation with stewardship and science with sustainability. By continuing to refine these methods responsibly, society can ensure that the tools of modern biotechnology contribute to long-term food security and ecological balance for living organisms on Earth.

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Acute stress drives structural synaptic plasticity imbalance in the mesocorticolimbic system to promote anxiety

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Abstract

Acute stress can rapidly reshape brain circuits that control emotion, motivation, and decision-making. These changes occur through a process called synaptic plasticity, where the strength and structure of synapses adjust in response to stimuli. This paper explores how acute stress alters molecular and structural synaptic plasticity in the mesocorticolimbic system--a brain network that includes the prefrontal cortex, amygdala, nucleus accumbens, and the ventral tegmental area. When stress causes an imbalance between these regions, emotion-regulated areas become overactive while regulatory regions weaken, which contributes to anxiety. By understanding the molecular signals and structural adaptations that drive this imbalance, researchers can identify new ways to restore healthy brain plasticity and reduce anxiety through different types of treatments.Acute stress drives structural synaptic plasticity imbalance in the mesocorticolimbic system to promote anxiety Acute stress and anxiety can rapidly change brain circuits involved in emotion, motivation, judgement, and memory, which in turn strongly impacts behavior and wellbeing. These broad neurocircuitry changes originate at the synapse, where neurons communicate with each other to facilitate cognitive functions. The process of synaptic plasticity, where synapses either strengthen or weaken in response to changes in neuronal activity, depends on both molecular signals and structural features, and significantly contributes to neuronal function3. The mesocorticolimbic system, which includes the prefrontal cortex, amygdala, and nucleus accumbens, is especially affected by stress1. This pathway helps regulate emotion and reward, and when its plasticity is disrupted, it can contribute to anxiety disorders. While researchers have studied the impact of stress on plasticity, fewer studies have observed the role of plasticity in the development of acute stress and subsequent systemic effects. Anxiety disorders are very common, thus it is important to understand how short term stress shapes the brain. The purpose of this paper is to elucidate how acute stress and anxiety affect molecular and structural synaptic plasticity in the mesocorticolimbic system, and how plasticity shapes neurocircuitry to exacerbate anxiety. These acute stress driven changes may explain how anxiety develops, and potentially point towards novel therapeutic strategies. Molecular Mechanisms of Synaptic Plasticity The brain is composed of many regions that are involved in coordinating complex thoughts, behaviors, and emotional processing. In order for the brain to facilitate complicated functions, regions must be connected into circuits made of neurons. These neurons communicate through synapses, which are the junctions where one neuron releases chemicals known as neurotransmitters that act on receptors of the recipient neuron. A basic rule is "neurons that fire together, wire together": where repeated coordinated activity makes synapses stronger, and continuous inactivity makes synapses weaker. This process, known as synaptic plasticity, is the brain's way of strengthening useful connections and weakening unnecessary ones. Synaptic plasticity is the process by which synapses, neurons, and neural circuits change to store and preserve information that shapes an organism's behavior4.

There are two common forms of lasting change: LTP (long term potentiation), which is durable strengthening of a synapse, and LTD (long term depression), which is durable weakening. LTP/LTD are driven by molecular and structural signaling cascades. The first form of molecular synaptic plasticity involves changes in glutamate receptor number on the surface of the postsynaptic neuron (AMPA and NMDA receptors). The second form is through growth or pruning of dendritic spines, which are the physical sites of excitatory synapses. Finally, the third process entails complex intracellular signaling cascades such as CAMKII, ERK/MAPK and immediate early genes (IEG) like c-Fos and Arc that drive protein interactions to stabilize change4.

Acute stress directly influences these molecular steps. Stress increases the synaptic concentration of neurotransmitters such as norepinephrine and dopamine, which act on receptors including beta-adrenergic receptors (beta-ARs). Hippocampal neurons express many beta-ARs, and hippocampal pyramidal cells have a high concentration of beta-adrenergic receptors, and activating those receptors strongly enhances hippocampal long-term potentiation (LTP)2. Stress-driven norepinephrine can lower the threshold for synapses to potentiate and therefore make LTP more likely. Stress also activates kinases like ERK, which is involved in supporting spine growth. Activation of ERK signaling increases dendritic spine density in the medial prefrontal cortex (mPFC), which supports better learning and memory4. Finally, acute stress triggers rapid IEG induction, which supplies the proteins needed to stabilize receptor and spine changes. In short: acute stress changes neurotransmitter levels, which then act on beta-ARs and other receptors, driving intracellular cascades and IEGs. In addition, modulation of AMPA/NMDA receptor density via endosomal trafficking and spine remodeling also contribute to shifts in LTP/LTD. These mechanisms direct the molecular route by which a single stressful event can begin to rewire circuits8.

The Role of the Mesocortolimbic System in Anxiety

Plasticity is essential for neural circuits to function, especially for complex emotional thinking and motivated behavior. The main circuit for these functions lies in the mesocorticolimbic system. Dopamine neurons in the ventral tegmental area (VTA) project to the nucleus accumbens (NAc) and prefrontal cortex (PFC), while the amygdala and hippocampus feed emotion and memory context into the loop. This network assigns value, drives motivation, and helps decide whether to approach or avoid situations -- all central to fear and anxiety. This population of cells forms the mesolimbic and mesocortical pathways, which are involved in reward (positive and negative reinforcement), assigning motivational value (incentive salience), aversion processing, and decision-making1.

Each node of the mesocorticolimbic system has a distinct role. The VTA is a small region in the midbrain that contains neurons that release dopamine. VTA dopamine neurons show increased activity after unexpected rewards, which motivates learning. These neurons also alter how much effort the brain might invest in to potentially receive another reward; VTA firing patterns therefore shape reinforcement learning and effort related decision-making10. The VTA connects to other parts of the brain like the NAc so different messages get spread. These dopamine signals have been recorded and shown they change with learning and effort1.

The NAc has a central role in motivated behavior. The NAc sits near the middle of the brain and acts like a control hub that helps turn wanting into doing. The NAc receives glutamatergic inputs from the mPFC, amygdala, and hippocampus9, whereas the NAc receives dopaminergic input from the VTA. The NAc integrates these inputs and decides whether an action is worth the work, which will determine whether you feel more approach or avoidance.

The PFC is positioned at the front of your brain and drives decision-making and executive function. The PFC controls critical thinking and communicates to other regions of the brain, such as the NAc and VTA, what action to take; recent imaging and circuit-mapping studies also show distinctive mesocorticolimbic alterations across psychiatric disorders that involve PFC dysfunction7.

The amygdala is the brain's processing center for emotions, especially fear. When the amygdala senses a threat, it initiates rapid physiological responses, including elevated heart rate and hyperventilation. It also helps store emotional memories for future recall in response to anxiety-inducing stimuli. Imbalance between the amygdala and mPFC can cause overreaction to certain cues or inability to calm down, core features of anxiety3.

The hippocampus encodes memories and supplies memories with situational context. The interaction of the hippocampus with the amygdala allows for the linking of memories of an event with emotion. Additionally, the hippocampus connects to the mPFC, adding memory and emotional recall to processing, judgement, and

subsequent behaviors. Thus, the interconnection of these regions contributes to the mesocorticolimbic system and influences how memory, judgement, and emotion are combined into anxiety-driven behavior.

The Impact of Acute Stress on Synaptic Plasticity in the Mesocorticolimbic System

Importantly, stress does not affect every brain region the same way. Repeated findings show a regional pattern, where emotion and reward areas such as the amygdala, NAc, and parts of the VTA tend to gain spines and become more excitable after stress, while cognitive and regulatory areas like the mPFC and parts of the hippocampus become less excitable and show impaired synaptic potentiation. Stress increases spine density and causes hyperexcitability in amygdala and NAc neurons, while it decreases spine density and impairs LTP in pyramidal neurons of the PFC and hippocampus3. Animal experiments support these findings, such that mice vulnerable to social stress show reduced spine density in the mPFC and increased spine density in the NAc and VTA9. These shifts in physical connectivity mirror functional changes -- the circuits that drive fear and motivated behavior become more connected and easier to activate, while circuits that provide top-down control and flexible thinking are weakened5.

Part of the reason that regions differ is due to receptor and neurotransmitter distribution. The hippocampus, PFC, and amygdala express higher densities of beta-ARs, or glucocorticoid receptors, so they respond differently to the same stimuli. Local growth factors such as BDNF and other signals can further direct dendritic spines to grow or shrink. Also, the same stress input can change the balance of LTP versus LTD at different synapses: in some hippocampal synapses a single stressor actually lowers the threshold for potentiation, making LTP easier to induce, while other hippocampal synapses that are in the same circuit undergo AMPA receptor internalization and weakening. For instance, one study found that a single session of stressful two-way active-avoidance conditioning (which means a behavioral task where an animal learns to prevent an aversive stimulus by moving to the opposite side of a cage in response to a warning signal) enhances LTP and lowers the activation threshold for an LTP that depends on beta-adrenergic receptors and NMDA receptors2. This shows that even one acute event can prime certain synapses to be more easily strengthened later while simultaneously causing others to weaken .

Timing of these coordinated signals matters. Receptor trafficking and kinase activity can occur within minutes to hours of activation, as well as IEG induction and new protein synthesis4. However, spine remodeling can persist for days to weeks of an acute stress event8. Due to the varying temporal response periods of the molecular mechanisms underlying plasticity, a single acute stressor can cause rapid cellular changes while also leaving a lasting mark in the circuit. These can ultimately strengthen emotion/reward pathways while weakening regulatory pathways, which produce the network imbalance observed in anxiety. In short, acute stress drives molecular cascades that remodel spines differently across regions, and those structural changes are a core reason why stressful experiences can produce persistent shifts toward anxiety-like behavior.

Acute stress shifts plasticity within this circuit in ways that promote anxiety. Mechanistically, stress-driven increases in dopamine and norepinephrine alter receptor trafficking and intracellular signaling in the mesocorticolimbic nodes. For example, higher dopamine from the VTA can alter AMPA/NMDA ratios in the NAc and PFC, changing how strongly those synapses respond. Beta-adrenergic signaling (beta-AR activation) from norepinephrine can enhance LTP-like changes in the hippocampus and influence PFC plasticity2. The ventral subiculum of the hippocampus is a key stress integrator. The ventral subiculum coordinates the stress response because it provides feedback control over the hypothalamic-pituitary-adrenal (HPA) axis and is densely innervated by norepinephrine2. This means that hippocampal outputs can increase neurotransmitter release and therefore change plasticity across the mesocorticolimbic network.

Functionally, these molecular changes cause region-specific shifts. Emotion and reward nodes (amygdala, NAc) feature overstrengthened synapses (more AMPA, more spines), while regulatory nodes (mPFC, dorsal hippocampus) often show weakened plasticity (less AMPA, spine loss). This imbalance in logical and

emotional processing is how acute stress-triggered molecular changes in synaptic plasticity translate into anxiety-like behavior.

Conclusion

Individual synapses experience the molecular effects of acute stress first, which ultimately contribute to widespread neural network changes that leave the mesocorticolimbic system vulnerable to future acute stress. When stress occurs, bursts of neurotransmitters change how receptors localize at the postsynaptic membrane and activate signaling molecules like ERK, PKA, and CaMKII, as well as IEGs. Over minutes to hours, receptors get redistributed, kinases activate signal cascades, and IEGs are rapidly transcribed. Over hours to days, IEGs drive protein production and reshape dendritic spines, turning short-term signals into longer-lasting changes in synaptic strength.

These local changes connect to larger systems like the mesocorticolimbic network (VTA, NAc, PFC, with help from the amygdala and hippocampus). Since different brain areas have different receptor types, the same stress signals can have opposite effects. Emotional and reward regions (amygdala, NAc) tend to grow more spines and become more excitable, while control regions like the mPFC and hippocampus often lose spines and weaken signaling. Therefore, acute stress-driven molecular shifts in plasticity create systemic imbalances that leave the brain vulnerable for future anxiety. This imbalance often manifests as hyperexcitability in emotion-related regions like the amygdala, while logical control centers become less responsive--amplifying the brain's sensitivity to future anxiety-provoking stimuli.

These shifts change how the brain processes information: emotional circuits become stronger and more reactive, while control circuits weaken. This imbalance helps explain why short bursts of stress can lead to lasting anxiety like behaviors. Clinically, this suggests treatments should focus on restoring healthy plasticity by adjusting receptor activity, using behavioral training during key plasticity windows, or rebalancing network excitability. Understanding how stress moves from cells to circuits helps explain why one stressful event can have long term effects and how we might reverse them.

Clinically, this points to two ways to restore balance: behavior interventions timed to plasticity windows (like exposure therapy or targetive cognitive training) and medications that either reduce hyperexcitability or promote healthy plasticity. Common drugs include SSRIs/SNRIs, which raise serotonin and sometimes norepinephrine6. Over weeks, these medications can support synaptic growth. Benzodiazepines can quickly enhance GABAergic inhibition to calm hyperactivity but carry dependency risks. Buspirone is a slower-acting 5-HT1A option for generalized anxiety, and beta-blockers are commonly used short-term for physical symptoms. Taken together, medications can either dampen excessive circuit activity or help create conditions that favor repair and resilience. Understanding the cellular mechanisms of anxiety will support future therapeutic development that can improve quality of life for affected individuals.

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Applying Classification and Regression Machine Learning Models to Detect AIDS-affected Patients

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Abstract

Acquired Immunodeficiency Syndrome (AIDs) is a chronic and most serious stage of human immunodeficiency virus (HIV), and remains one of the largest public health challenges globally. HIV is characterized by weakened immune systems, infecting white blood cells, called CD4 cells or helper T cells, causing your white blood cell count to drop. This ultimately results in vulnerability to illnesses that, when left untreated, can progress into AIDs. Currently, traditional diagnostic and prognostic assessments of AIDS progression often rely on manual analysis of patient data, which can be time-consuming, inconsistent, and dependent on human expertise. This exploratory paper aims to apply machine learning methodologies to predict AIDS infection status using patient demographic, medical history, treatment, and immunological data derived from the AIDS Clinical Trials Group Study 175 dataset. Models were trained using multiple machine learning algorithms, including logistic regression models, decision trees, random forests, and XGBoost, and evaluated based on numerous evaluation metrics such as precision, accuracy, and confusion matrices. By integrating traditional machine learning methodologies into AIDs detection, this research aims to enhance diagnostic precision, increase data efficiency, and support clinicians in developing more effective treatment strategies for individuals affected by HIV/AIDS.

Keywords: AIDs, HIV, Random Forest, Decision Trees, Classification Models, Linear Regression, Machine Learning

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Introduction

Acquired Immunodeficiency Syndrome (AIDs) is a chronic and most serious stage of human immunodeficiency virus (HIV), and remains one of the largest public health challenges globally. HIV is characterized by weakened immune systems, infecting white blood cells, called CD4 cells or helper T cells, causing your white blood cell count to drop. This results in vulnerability to illnesses that, when left untreated, can progress into AIDs.

Currently, HIV/AIDs is a global epidemic that continues to rise sharply. Amplified by globalization, HIV/AIDs has continued to spread at an accelerating rate. Most recent data from the Joint United Nations Programme on HIV/AIDS (UNAIDS) reports that approximately 40.8 million people globally were living with HIV in 2024, with 630,000 people dying from AIDS-related illnesses in that same year ("Global HIV & AIDS statistics," 2025). Despite growing access to funding, interventions, and technical support, many low-resource

regions continue to face rising infection rates (Coovandia & Hadingham, 2005). Although current methods have generally slowed the growth of HIV/AIDs, the most recent projections by a team of hundreds of GBD 2021 HIV Collaborators indicate that existing levels of HIV control are insufficient to meet global incidence and mortality reduction targets by 2030. Only a few countries will meet the 2030 target for reducing HIV incidence and HIV-related deaths by 90% from 2010 levels. Additionally, over 40 million individuals worldwide will likely continue to depend on lifelong antiretroviral therapy (ART) for decades to come (Carter et al., 2024). These findings reveal the urgent need for sustained global cooperation, innovative approaches, and scalable technologies to accelerate progress toward ending AIDS as a major public health threat.

Despite significant advances in treatment and prevention, early detection of HIV remains a major challenge, especially within low-resource settings. Although viral load testing continues to expand rapidly, potentially reaching nearly 30 million tests by 2024, CD4 testing demand continues to decline despite being critical for patients initiating treatment or in areas with limited access to viral load testing. (World Health Organization, 2022). A continued decline in availability for CD4 testing creates a gap in available detection methodologies for patients with more advanced HIV diseases, possibly delaying treatment initiation and worsening outcomes for families. Furthermore, HIV screening strategies in emergency departments are facing stark challenges of efficient detection: in a trial of 76,561 patient visits, only 0.2% of tests identified new HIV diagnoses, with costs per new diagnosis ranging from $18,762 for traditional targeted screening to $23,554 for enhanced targeted approaches (Haukoos et al.2024). Enhanced screenings in high-volume settings yield fewer diagnoses and require high costs, highlighting the need for more targeted, efficient, data-specific approaches in classifying and identifying HIV/AIDs within patients, especially in supporting lower-resource countries.

To address these challenges, researchers have already increasingly turned to data-driven and machine learning methods to improve HIV detection and optimize patient classifications. By using machine learning algorithms, scientists can analyze and find correlations between patient demographics, clinical history, and treatment records, to possibly predict disease progression and whether patients may have HIV/AIDs. Machine learning has shown promise in predicting HIV risk, particularly when also detecting STIs. Xu et al. (2022) developed a web-based tool that predicted HIV (AUROC = 0.72) and other STIs, displaying future capabilities of data-driven risk assessment to guide testing and prevention in high-risk populations, especially in resource-limited settings. Mutai et al. (2021) applied a machine learning architecture called XGBoost to analyze HIV Impact Assessment (PHIA) data for 41,939 male and 45,105 female respondents with 30 and 40 variables within sub-Saharan countries. Furthermore, Marcus et al. note that ML can find patterns in large datasets to better target prevention, but challenges remain with bias, validation, and implementation in low-resource settings.

Despite the potential of machine learning, most existing research papers examine and optimize a single specific algorithm in identifying AIDs/HIV. In contrast, our research evaluates multiple machine learning approaches, ranging from logistic regression models, decision trees, random forest models, and XGBoost, comparing their performance on predicting HIV infection and associated risk factors. Our goal is to expand on past machine learning detection papers while identifying the most accurate and scalable models for resource-limited settings for future technologies.

Materials/Methodology/Models

Dataset Preprocessing

This research utilized the AIDS Clinical Trials Group Study 175 Dataset from the UCI Machine Learning Repository (Hammer et al., 1996). This dataset contains 50,000 synthetic records with various attribute information with respect to patients. The attribute information and their definitions are displayed in the table below:

Table 1: AIDs Dataset Variables and Respective Attribute Information for Baseline Research Understanding

As evident from the detailed table above, this dataset is filled with a rich and comprehensive set of clinically relevant variables. Thus, this dataset was selected to allow for meaningful analysis, flexibility when creating machine learning models, and better correlating variables and their effects on model outcomes.

Dataset Preprocessing: Min-Max Scalers/Train Test Split

In order to continue to preprocess the data, we must normalize the data with Min-Max scaling. This process was necessary as an issue with machine learning models is that when given numeric values, larger values may influence the decisions more so than smaller values, even if those values aren't inherently more important For example, if one feature represents age (ranging from 0-100) and another represents annual income (ranging from 20,000-200,000), the model may focus more on income simply because of its larger magnitude. Min-Max Scaling helps to solve this problem by rescaling each feature to fit within a specific range (0-1). Min-Max Scaling ensures that each feature contributes equally to the model, preventing features with larger raw values from dominating. The transformation is shown in the equation below:

Where in this equation:

X represents the original value of a feature for a singular data point

min(x) represents the minimum value of that feature across the entire dataset

max(x) represents the maximum value of that feature across the entire dataset

X' represents the transformed value of the feature

Within Python, the sklearn library is able to perform min max scaling at a large scale on an entire dataframe. Utilizing this, we are able to transform our data in its entirety, applying this min-max scaler to every single datapoint. Our code is visualized and explained in the section below:

Line 1: Import the MinMaxScaler from the scikit-learn library. This built-in scaler allows us to easily rescale numeric values so that every column of our dataset falls between 0 and 1.

Line 3: Create an instance of the MinMaxScaler and store it in the variable scaler. This object will calculate the minimum and maximum values of each feature (column) and use them to apply the scaling transformation.

Lines 5-7: Apply the scaler to the entire DataFrame df. The fit_transform() method first learns the min and max of each feature (fit) and then rescales the data (transform). Wrapping the result in pd.DataFrame() ensures that the scaled data is returned as a DataFrame with the same column names as the original.

Line 9-10: Line 9 prints the first five rows of the original dataset using df.head() so we can compare the raw values against the transformed ones. Lines 10 print the first five rows of the scaled dataset df_scaled. At this point, each feature has been rescaled between 0 and 1, ensuring that no column dominates another due to its larger numerical values.

After applying Min-Max Scaling changes to our dataset with entirely numerical ranged data, this dataset is set up well for binary classification models without the need for excess data cleaning and correlation maps. From here, we used Sklearn train_test_split to divide the model into training and testing dataframes for evaluating our model. Our code is visualized and explained below:

Line 1: import the essential libraries needed to create this linear regression model. train_test_split: splits data into training v.s. testing datasets.

Lines 3-4: create the X matrix and y variable for the model. The X matrix represents all of the features/independent variables that will be used by the regression model to make predictions. The y variable is dependent on this X matrix data to learn and is the target variable/what the model is trying to predict.

Line 5: Uses train_test_split to split the dataset into 80% training and 20% testing data. The training data will be the data that is used to fit the model while the testing data will be used to later evaluate the model performance. The random_state of 42 is a set variable that ensures the same randomness every runtime, so that results are ensured to be reproducible.

Exploratory Data Analysis

Before beginning the development of our machine learning models, several exploratory data analysis steps were applied to better track data trends/patterns, ensure data consistency, and address class imbalances if necessary. This exploratory data analysis section was built upon a fully synthetic dataset that had additional test cases synthetically implemented. All modeling was done with the normal subset with non-synthetic data. This was done as synthetic testing helps to establish general controlled patterns within the dataset. After establishing generalized patterns, we could apply this to our modeling process and better interpret the results of our model.

To best determine data consistency and identify trends/patterns, a strategic data visualization was created to identify outliers and trends within features. The section below details the respective data graph and a brief explanation of what pattern it teaches us:

Graph 1: Age Distribution by Infection Status

The rationale behind Graph 1 was identifying whether or not patients being affected by AIDs had any stark correlation to their Age. If the infected status was skewed right or left with age, this could have possibly shown us a stronger correlation between cases right away. However, as visualized in the graph above, since the majority of patients were between the ages 30 - 40, the resulting patients affected by AIDs were at a similar spread. Therefore, there is no significant correlation we can come up with for age and AIDs infection status from this graph.

Graph 2: Gender vs Infection Status

Graph 2 compares the count of infected patients to non infected patients by gender. By being able to compare differences in infected/non-infected status, it is possible to identify if one gender had a drastically larger population affected, being able to detect a possible pattern in Male/Female patients. However, since the count for infected for both Male and Female was approximately 50% of the non-infected count, we can assume that there is no significant trend in male vs female and the correlating infection status.

Graph 3: Race vs Infection Status

Graph 3 rationale is similar to Graph 2 in the sense that by comparing the infected/infected status between race, it is possible ot identify if being white or non-white significantly factors into your infection status. However, considering that the amount of infected cases is approximately 45% of the non-infected status for both white and non-white individuals, we can assume that race does not significantly factor into the correlated infection status.

Graph 4: (Left) CD8 T-cell Count for Infected and Non-Infected at Baseline. (Right) CD8 T-cell Count for Infected and Non-Infected after 20 weeks

Graph 4 represents two different visualizations comparing the CD8 T-cell counts between infected na dnin-infected individuals. The left displays the baseline and the right measures the T-cell count after 20 weeks. From both graphs, the T-cell counts appear to be relatively similar suggesting no initial difference.

Logistic Regression Model Mathematical Theory

After setting up our dataset, we began with our first machine learning model: the Logistic Regression Model. A Logistic Regression model is a supervised machine learning algorithm commonly used for binary classification problems (tasks defined by the goal being to categorizing data into one or two distinct classes). A logistic regression model is useful for our research as we can bound our results between 0 (not having AIDs) and 1 (having AIDs), coming up with predictions based on estimating the probability that a given input belongs to that class.

A Logistic Regression model is built based on a linear combination score. This linear combination score is essential, providing a single numerical score summary of how strongly those features suggest a person has AIDs or not AIDs based upon the dataset. A linear combination score can be represented mathematically as:

Where in this equation:

Values of x are the input features. These are the specific patient attributes being input, such as age, CD4 count, etc.

Values of w are the model weights. These determine how much influence each feature has on the prediction. For example, if the weight for one variable was 0, then it would be entirely excluded from making the prediction.

Variable b is the bias term. This adjusts the model's output independently of the features. The bias allows the model flexibility to better fit the data.

The resulting linear combination score is z, providing a continuous score where a negative value means less likely to have AIDs while a larger positive value means more likely.

An issue with using a pure linear combination score is the value. There are no upper or lower bounds, making it hard to determine a pure probability interpretation just using this possibly very large or low number. Thus, a sigmoid function is next utilized, converting data inputs into a value between 0 and 1, making it ideal for expressing probabilities. The function is defined below:

Where in this equation:

When z is a very large and positive value, approaches 0, meaning the function approaches 1.

When z is a very large and negative value, approaches a large value/infinity, so the function approaches 0.

If z is a low value near 0, the function is approximately 0.5.

When graphing this sigmoid function, it bounds the output between 0 and 1, allowing for the output, , to represent a decimal that can be used as an estimated probability. The closer the output is to 1, the higher the model's estimated probability that the input belongs to the positive class (having AIDS), and the closer to 0, the lower the probability.

Now that we have our probability, we need a way to train the model over time and adjust its weights for the linear combination score. This is where Binary Cross-Entropy Loss (BCE) factors in. Also called log loss, BCE, measures the "distance" between the predicted probability distribution and the true labels. Using alternative loss functions, such as Mean Squared Error (MSE), isn't ideal here because it can produce poorly shaped gradients near 0 or 1, which is important for our logistic function. The function for BCE is defined below:

Where in this equation:

represents the ground truth (0 or 1)

represented the predicted probability of the value from the sigmoid function

When y = 1, the loss becomes , therefore, the closer becomes to 1, the smaller the loss is.

When y = 0, the loss becomes , therefore, the closer becomes to 0, the smaller the loss is.

This formula helps to penalize confident wrong predictions more heavily than less confident ones using the log function, helping to tune the weights and predictions accordingly. When applying this across a full dataset of N total samples, BCE can be represented in a similar way, now finding the average of the total loss calculations to help the model learn by a defined loss factor. The function is defined below:

Where in this equation:

represents the ground truth (0 or 1) specifically for sample i

represented the predicted probability of the value from the sigmoid function specifically for sample i

represents the sum of the loss terms

is the total number of samples within the dataset

During training, the model repeatedly iterates over the dataset for multiple epochs. During which, the model calculates the average BCE loss over all samples and then updates the model's weights and bias using an optimization algorithm. One specific kind of optimization algorithm is gradient descent. Over successive epochs, these updates gradually improve the model's predictions, producing probabilities closer to the true labels and reducing the BCE loss.

A simpler way to implement BCE and training a machine learning model is deriving both the sigmoid function and the BCE equation. By deriving both of these functions/equations, we are able to generate a simpler gradient formula () which can simply be used to update the model's weight and biases overtime. This avoids complex computations above, while also making epochs slightly more efficient but doing the same training transformations overtime. The derivation process is show below:

By combining the sigmoid and BCE derivation, logistic regression becomes mathematically efficient for probability-based predictions assisting us in our research of identifying AIDs in patients based on clinical and demographic features.

Logistic Regression Model Programming Application

Now having derived the sigmoid function and Binary Cross-entropy loss functions, combining them into an efficient gradient function for weight training, we can now apply these concepts into our research through Python. The scikit-learn library provides a built-in implementation of logistic regression, handling the sigmoid function, the BCE loss, and performs gradient-based optimization to fit the model. This assist our research in being more structured and efficient, calculating predictions, computing loss, and updating weights. Our code is visualized and explained below:

Line 1: Import the necessary libraries. LogisticRegression is the model we will use for classification. accuracy_score, confusion_matrix, and classification_report are metrics that help us evaluate the performance of the model.

Lines 3-4: Split the dataset into training and testing sets using train_test_split. test_size=0.2 reserves 20% of the data for testing and 80% for training. stratify=y ensures that the proportion of classes in the target variable is maintained in both training and testing sets, which helps avoid bias from class imbalance. random_state=42 makes sure the split is reproducible each time the code is run.

Lines 6-8: These lines help initialize and train the logistic regression model. log_reg = LogisticRegression(max_iter=1000) creates the model and allows up to 1000 iterations for the solver to converge, which is helpful to have more iterations for better training over time. log_reg.fit(X_train, y_train) trains the model on the training data, learning the coefficients for each feature and training the model. y_pred = log_reg.predict(X_test) uses the trained model to predict the target values for the test set.

Lines 10-12: Evaluates the performance of the trained model. model.accuracy_score(y_test, y_pred) measures the accuracy or the proportion of correct predictions.confusion_matrix(y_test, y_pred) provides a detailed breakdown of true positives, true negatives, false positives, and false negatives. classification_report(y_test, y_pred) gives precision, recall, F1-score, and support for each class, which is especially important when dealing with imbalanced classes. This allows us to better see how the model performed.

Decision Tree Model Mathematical Theory

Our second machine learning model tested was a simple Decision Tree Model. Decision trees are a type of supervised learning models that can be used for both classification and regression tasks, operating through a series of binary decisions that split data step by step from featured values.

The process begins at the root node, which contains the full dataset and represents the first decision point. From there, the model evaluates which feature and threshold best separates the data into distinct classes using criteria such as Gini Impurity or Shannon Entropy. Gini Impurity functions by measureing how mixed the classes are in a node, while Shannon Entropy quantifies the uncertainty of the dataset, similar to the metric used in logistic regression.

Internal nodes are nodes that continue to get splitted until all data is properly split into groups. These splits all occur recusively without putting previous splits into consideration. These series of splits are repeated indefinitely until the data is separated into leaf nodes representing final predictions. The leaf nodes represent the final outcomes of the tree, assigning a predicted class label for the data points that reach them.

Figure 1: A Sample Decision Tree Node Breakdown ("Decision Tree in Machine Learning," 2024)

One limiting factor of Decision Tree Models is their proneness to overfitting data. Overfitting occurs when a machine learning models does not learn the underlying pattern and correlation between the data properly, meaning it is only suitable for the training data, but other datasets it performs underwhelmingly. Decision Trees are naturally prone to overfitting due to their overall structure and architectural design leading to issues listed below:

Greedy Splitting: At each node, the tree chooses the feature and threshold that best separates the training data without considering future splits or overall tree structure. This results in overfitting when looking at other data.

Deep Trees Can Factor in Outliers: If the decision tree is allowed to grow indefinitely or for a long time, it can repeat until leaf node perfectly classifies the training data. However the deeper the tree is, this leads to very specific combinations of features being divided including noise or outliers, which will lead to overfitting data.

Decision Tree Model Programming Application

Now discussing the theoretical backbone of Decision Trees and their functionality, we can now apply these concepts into our research through Python. The sci-kit learn library provides a built-in implementation of decision trees, allowing us to easily fit our model to our dataset. We can better integrame handling of nodes, split criteria, and generating results. Our code is visualized and explained below:

Line 1: Import DecisionTreeClassifier from scikit-learn. This model creates a tree-based classifier that splits data into branches based on feature values to make predictions.

Lines 3-6: Initialize and train the decision tree model. dt_model = DecisionTreeClassifier(random_state=42) creates the model and ensures reproducibility by setting a fixed random_state. dt_model.fit(X_train, y_train) trains the model on the training data, learning how to split the features to classify the target variable. y_pred_dt = dt_model.predict(X_test) uses the trained model to predict the target values for the test set.

Lines 8-10: Evaluate the performance of the trained decision tree. accuracy_score(y_test, y_pred_dt) measures the proportion of correct predictions. confusion_matrix(y_test, y_pred_dt) shows the breakdown of true positives, true negatives, false positives, and false negatives. classification_report(y_test, y_pred_dt) provides precision, recall, F1-score, and support for each class, giving a detailed performance overview.

Random Forest Model Mathematical Theory

Our third machine learning model tested was a Random Forest Model, a machine learning model utilizing a multiple tree architecture to better learn and train testing models. As mentioned earlier, a Decision Tree can be prone to overfitting due to noise or outliers in the training data and not factoring in past layers. A Random Forest Model helps to mitigate thes issues by having numerous predictions of trees each trained on different, smaller subsets of the data. A random forest model works by:

Bootstrapping: For each tree, a random subset of the training data is selected, helping to make diversity amongst the trees

Random Feature Selection: At each split in a tree, only a random subset of features is considered when determining the best split. This helps to prevents trees from becoming too similar.

Averaging: At the end of predictions for each tree, they output a respective class prediction. The Random Forest assigns the final class based on majority vote

Figure 2: A Simplified Diagram of a Random Forest Model (Koehrsen, 2017)

Random Forest Model Programming Application

Now discussing Random Forest models and how they function, we can now apply these concepts into our research through Python. The sci-kit learn library provides a built-in implementation of a Random Forest Model, allowing us to easily fit our model to our dataset. We can better integrame handling of nodes, split criteria, and generating results. Our code is visualized and explained below:

Line 1: Import RandomForestClassifier from scikit-learn. This model creates an ensemble of decision trees and combines their predictions to improve accuracy and reduce overfitting.

Lines 3-5: Initialize and train the random forest model. rf_model = RandomForestClassifier(n_estimators=100, random_state=42) creates a model with 100 trees and ensures reproducibility with a fixed random_state. rf_model.fit(X_train, y_train) trains all 100 trees on random subsets of the training data and features, allowing the forest to generalize better than a single decision tree. y_pred_rf = rf_model.predict(X_test) uses the trained forest to predict the target values for the test set.

Lines 8-10: Evaluate the performance of the trained random forest. accuracy_score(y_test, y_pred_rf) measures the proportion of correct predictions across the test set. confusion_matrix(y_test, y_pred_rf) shows how predictions break down into true positives, true negatives, false positives, and false negatives. classification_report(y_test, y_pred_rf) provides precision, recall, F1-score, and support for each class, offering a detailed view of the model's performance.

XGBoost Model Mathematical Theory

The last machine learning architecture we tested was boosted tree methods with an XGBoost model. Within an XGBoost model, trees are built sequentially to correct the errors of previous trees. Each leaf node in a tree has a weight that determines its contribution to the prediction, allowing for the XGBoost model to learn and adjust from each epoch iteration.

Figure 3 : Example diagram of a Boosted Tree Model minimizing error over more epochs

Unlike a single Decision Tree or Random Forest, XGBoost optimizes a function that combines training loss with a process called Regularization, helping prevent overfitting while improving predictive performance. Regularization adds a "regularization" term to the model's loss function, reducing overfitting and improving predictive performance overtime. The two regularization methods used are L1 (Lasso) and L2 (Ridge) regularization, assisting the model behavior.

L1 Regularization (Lasso) is a regularization method that adds a penalty to the loss function to prevent overfitting. This penalty is based on the absolute values of the coefficients, which is different from Ridge and other regualrization techniques. Lasso Regularization can be represented mathematically as:

Where for the first term (Loss Function):

represents the actual target value for the sample i

represents the feature j within the data sample i

represents the respective weight for feature j

represents the predicted value for sample i bbased upon the model

Where for the second term (Regulatization Term):

represents the regularization hyperparameter that helps control the strength of the regularization term

represents the absolute value of the weight for the respective feature j.

adds a regularization term proportional to the magnitude of weights. This can shrink weights to 0 removing unnecessary and unimportant features as needed

Within this equation, multiple calculations are being performed in order to better improve the respective model. The first term, a Loss Function measures how well the model's predictions matched the original outcomes. For every model, the objective is to minimize this term as best as possible, to ensure that the model fits the training data as accurately as possible. The Regulatization Term added by the L1 Regularization addresses overfitting of the loss on the training data, by adding a penalty that is proportional to the absolute value of the model's coefficients. This penalty helps by shrinking some weights for classes to 0, removing them from the prediction process. This allows Lasso Regularization to do feature selection, allowing for different values to be tested overtime rather than just having a board generalization of factors. This plays into the Random Forest model as over the training epochs, by having Lasso regularization conduct feature selection, certain patterns and its importance in model performance can be better correlated, increasing the efficiency and metrics of the model.

L2 Regularization (Ridge) is a regularization method that adds a regularization term to the loss function to prevent overfitting. This method is different from L1 Regularization as rather than possibly removing some features, all features stay the same, but the size of the data shrinks as well. Ridge Regularization can be represented mathematically as:

Where for the first term (Loss Function):

represents the actual target value for the sample i

represents the feature j within the data sample i

represents the respective weight for feature j

represents the predicted value for sample i bbased upon the model

Where for the second term (Regulatization Term):

represents the regularization hyperparameter that helps control the strength of the regularization term

represents the squared value of the weight for the respective feature j.

adds a regularization term proportional to the magnitude of weights. This can shrink weights to 0 removing unnecessary and unimportant features as needed

Within this equation, multiple calculations are being performed in order to better improve the respective model. The first term, the same Loss Function as the L1 equation measures how well the model's predictions matched the original outcomes. The Regulatization Term added by the L2 Regularization addresses overfitting of the loss on the training data differently than L1, by adding a term that is proportional to the square of the model's coefficients instead of the absolute value. The importance of this squared term is that is shrinks all of the coefficients towards zero, but will never set any of them exactly to 0. As a result, Ridge Regularization factors in all of the features in the model which can be useful for Data Scientists for respective problems on how every variable factors into a model and if all features may play a part in the model. However, by lowering all of the variables, it controls overfitting not having too excessively large coefficient weights. For XGBoost, Ridge Regression can prevent trees from producing too extreme weights and shrink all weights proportionally, while also maintaining general patterns across all variables.

Figure 4: Geometrical Interpretation of L1 Ridge Regularization (Left) and L1 Lasso Regularization (Right). The ellipses represent the model's predictions without regularization, while the circle and paralelogram show the constraints added by regularization. For Ridge Regularization, this limits the squared magnitude of weights, keeping all weights small, forming a circular shape. Lasso Regularization limits the absolute values of weights, with its corners indicating that some weights become exactly zero, performing feature selection. ("Ridge and Lasso: Geometric Interpretation", 2019)

Now, these same concepts of regularization can be applied in understanding XGBoost and its mathematical applications. The general form of XGBoost over an iteration total of variable t is:

Where in this equation:

represents the ground truth value for the sample i.

represents the predictions of the trees built up over the iterations t-1

represents the new tree that is added at iteration of t

represents the regularization term for the new tree

For , the regularization term for the new tree can be defined as a combination of Lasso Regularization and Ridge Regularization. In addition, a penalty term is implemented to reduce the number of leaf nodes, keeping each tree smaller and simpler over iterations:

Where in the function:

represents the number of total leaf nodes in the tree

represents the weight score assigned to the leaf in j

represents the penalty applied to the total number of leaves. This encourages simpler trees.

represents L2 Regularization (Ridge), which penalized larger leaf weights and shrink them towards 0

represents L1 Regularization (Lasso) which may push weights on some classes to 0, removing them from contributing to the prediction.

As displayed within these equations, XGBoost is built upon two separate terms,the loss function and a regularization terms that incorporates both regularization methods (Lasso and Ridge). The first term ensures that each new tree added at iteration t improves the prediction by minimizing the difference between the true values and the ensemble's predictions so far. Essentially, this is how XGBoost "learns" from errors in prior

iterations, gradually improving its predictive performance. The regularization term incorporates both L1 (Lasso) and L2 (Ridge) penalties, as well as a penalty for the number of leaf nodes. This term is critical because it prevents overfitting, which is a common issue in tree-based models where deep or overly complex trees can perfectly fit the training data but fail to generalize. By controlling the number of leaves and shrinking or removing leaf weights, XGBoost maintains simpler, more generalizable trees. This is why XGBoost is highly effective for classification and regression tasks: it produces robust predictions while mitigating overfitting, making it especially useful in real-world datasets with noisy or high-dimensional features, such as in clinical or demographic data for disease prediction.

XGBoost Model Programming Application

Now discussing Random Forest models and how they function, we can now apply these concepts into our research through Python. The sci-kit learn library provides a built-in implementation of a Random Forest Model, allowing us to easily fit our model to our dataset. We can better integrame handling of nodes, split criteria, and generating results. Our code is visualized and explained below:

Line 1: Import the necessary library. xgboost is the library that provides the XGBClassifier class for building gradient-boosted decision tree models.

Lines 3-5: Initialize and train the XGBoost model. The model iteratively builds trees to minimize the loss function while using regularization (L1/L2 and leaf penalties) to prevent overfitting. y_pred_xgb = xgb_model.predict(X_test) uses the trained model to predict the target variable on the test dataset.

Lines 7-9: Evaluate the trained model. These metrics are particularly useful when classes are imbalanced, allowing a better understanding of model performance across different categories.

Hyper-parameter Tuning: Grid Search v. Random Search

Hyper-parameter tuning is a critical machine learning step that helps optimize respective models. Hyperparameters are set values prior to training that control the learning process of the model itself. Some examples of this are the depth of a decision tree, the total number of decision trees in a Random Forest, the set learning rate for an XGBoost model, etc.

Figure 5 : Graphical Representation of Grid Search (Left) and Random Search (Right). While Grid Search searches through a defined space and set of values equally, a random search iterates through sets of parameters sporadically ("Model Selection," n.d.)

There are two common approaches for hyperparameter tuning: Grid Search and Random Search. Grid Search is a hyperparameter tuning process that goes through every possible combination of hyperparameter within a pre-defined set of values. Once all the combinations are evaluated, the model with the set of parameters which give the top accuracy is considered to be the best. The limitations and advantages of Grid Search are shown below:

Table 2: Advantages and Limitations of a Grid Search tuning method

Within our research, the only model we implemented a Grid Search in was a Logistic Regression model, due to the lower amount of hyperparameters required to check in comparison to that of a Random Forest Model or an XGBoost model. There are numerous hyperparemeters that can be tuned to optimize a Logistic Regression Model:

C (Inverse of Regularization Strength): The C hyperparameter controls how strongly the model penalizes large coefficients. A smaller C applies stronger regularization, shrinking coefficients and helping prevent overfitting, though it may underfit if too small. A larger C allows the model to fit the training data more closely, which can improve accuracy on training data but risks overfitting to noise.

Penalty (Lasso / Ridge): The penalty hyperparameter determines the type of regularization applied to the model. 'L1' (Lasso) or 'L2' (Ridge) regularization can be applied to the Logistic Regression model.

Solver: The solver hyperparameter specifies the optimization algorithm used to fit the logistic regression model. Different solvers can handle different types of regularization and affect convergence speed. For example, 'liblinear' supports both L1 and L2 penalties and works well on smaller datasets, while other solvers may be better for large datasets or multiclass problems.

Now knowing these sets of variables, we can apply this to our research. Within the Sklearn library, Grid Search is already implemented so we can simply import it. Our code is visualized and explained below:

Line 1: Import GridSearchCV from scikit-learn. This tool allows us to systematically search for the best combination of hyperparameters for a given model using cross-validation.

Lines 3-6: Define a grid of hyperparameters to search. 'C': [0.01, 0.1, 1, 10] are the different values for the regularization strength (smaller C means stronger regularization). 'penalty': ['l1', 'l2'] is the types of regularization: L1 (Lasso) or L2 (Ridge). 'solver': ['liblinear'] is the algorithm used to optimize the logistic regression model; liblinear works with both L1 and L2 penalties.

Lines 9-10: Set up and run the grid search. grid_search = GridSearchCV(log_reg, param_grid, cv=5, scoring='accuracy'). log_reg is the base logistic regression model to tune. param_grid -> the hyperparameters to test. cv=5 is a 5-fold cross-validation, which splits the training data into 5 parts to ensure robust evaluation. scoring='accuracy' is the metric used to determine which hyperparameters are best. grid_search.fit(X_train, y_train) trains the model on every combination of hyperparameters and evaluates them using cross-validation.

Lines 12-13: Print the results of the grid search. grid_search.best_params_ shows the combination of hyperparameters that gave the highest cross-validation score. grid_search.best_score_ shows the best cross-validation accuracy achieved with those hyperparameters.

Within this Grid Search, our model will iterate through 8 total hyperparameter combinations checking each one for its best performance. Our results are shown in our results section.

The alternative hyper-parameter tuning method is a Random Search method. Random Search optimizes machine learning models by randomly sampling combinations from the specific set provided in the program. It will not exhaust every single option available. Rather, it will run for the number of iterations preset within the program. For each iteration, it will randomly select a value for each hyperparameter, train the model on the training set with those hyperparameters and then evaluate the performance with cross-validation. The limitations and advantages of Random Search are shown below:

Table 3: Advantages and Limitations of a Random Search tuning method

Now knowing these sets of variables, we can apply this to our research. Within the Sklearn library, Random Search is already implemented so we can simply import it. For our research, we utilized Random Search for our Random Forest Model and XGBoost model to cover wider ranges of values, shorten runtimes, and increase efficiency. Our code for our Random Forest Model is visualized and explained below:

Lines 1-2: Imports the RandomizedSearchCV function for randomized hyperparameter tuning and numpy for numerical operations.

Line 4: Defines the hyperparameter distribution set to sample from. This includes parameters such as the number of trees (n_estimators), tree depth (max_depth), minimum samples for splits and leaves, feature selection method, and whether to use bootstrap sampling.

Line 6: Initializes the Random Forest classifier with a fixed random_state to ensure reproducible results.

Line 8: Configures RandomizedSearchCV to sample 5 random combinations from the hyperparameter space, use 5-fold cross-validation to evaluate each combination, optimize for accuracy, and run in parallel

across all processors (n_jobs=-1).

Line 10: Fits the random_search object to the training data, which performs the randomized hyperparameter search and trains multiple Random Forest models.

Lines 12-16: Prints the best combination of hyperparameters found, make predictions on the test set, and evaluate performance using accuracy, a confusion matrix, and a classification report to understand precision, recall, and F1-score.

Our code for our XGBoost model is visualized and explained below:

Line 1: Define the hyperparameter search space for XGBoost. This includes the number of trees (n_estimators), tree depth (max_depth), learning rate, subsample ratio, column sampling ratio, regularization parameters (reg_alpha for L1 and reg_lambda for L2), and gamma (minimum loss reduction to make a split).

Line 3: Initialize RandomizedSearchCV for randomized hyperparameter tuning, sampling 10 random combinations from the defined hyperparameter space. It uses 3-fold cross-validation, optimizes for accuracy, prints progress (verbose=2), and runs computations in parallel (n_jobs=-1).

Line 5: Fit the randomized search object to the training data, performing model training for each sampled combination and selecting the best based on cross-validation accuracy.

Lines 7-9: Use the best hyperparameters found by RandomizedSearchCV to create a tuned XGBoost model (best_xgb). Fit this model to the training data and make predictions on the test set (y_pred_best).

Lines 11-15: Print the best hyperparameters, the best cross-validation accuracy from the search, and the performance on the test set using accuracy, confusion matrix, and classification report to evaluate precision, recall, and F1-score.

Results & Findings

In this section, we present our findings of our machine learning models when applying them to our AIDs dataset. Additionally, we examined how hyperparameter tuning through Grid Search and Randomized Search affected our model performance, highlighting the improvements achieved by optimizing model parameters. Our aim was to identify which models and configurations provide the most accurate and generalizable predictions, offering insight into the key factors influencing the target outcome.

Evaluation Metrics

Evaluation Metrics are important values that allow us to determine how well our models performed quantitatively. The evaluation metrics utilized to measure our models are: Accuracy, Precision, Recall, F1-Score, Confusion Matrices, and AUC/ROC Curves. An explanation overview of each metric is explained below.

Accuracy is a metric that measures the proportion of correct predictions out of all predictions made. It is a helpful overall metric that gives a relatively good idea of how often the model is correct when making its predictions. A drawback is that if the dataset is not equally balanced, accuracy may be inaccurate and not representative of the data well. Accuracy can be represented as:

Where in the expression:

TP represents the True Positives (correctly predicted positive cases)

TN represents the True Negatives (correctly predicted negative cases)

FP represents the False Positives (incorrectly predicted positive cases)

FN represents the False Negatives (incorrectly predicted negative cases)

Precision is an important metric that helps measure the proportion of correctly predicted positive cases out of all of the cases predicted as positive. Precision helps tell us how reliable our model is when it predicted the positive class. A higher precision means that when the model makes an assumption about the positive case, it is more likely to be correct, making it good for healthcare/clinical models where it is extremely important to be precise in what you evaluate. Precision can be represented as:

Where in the expression:

TP represents the True Positives (correctly predicted positive cases)

FP represents the False Positives (incorrectly predicted positive cases)

Recall is a metric that measures the proportion of the actual positives that were correctly predicted. Out of all of the cases that were predicted as positives (True Positives and False Negativees), how many were actually correct? Recall helps answer this question. Higher recall means that there is fewer false negatives, meaning that when it predicted positive it is likely not to mix it up with the other class. Recall can be represented as:

Where in the expression:

TP represents the True Positives (correctly predicted positive cases)

FN represents the False Negatives (incorrectly predicted negative cases)

F1-Score is an important metric which represents the harmonic mean of precision and recall. Thus, this metric helps provide a single metric balancing both of these values. This is useful when you need to identify balance between precision and recall when looking at imbalanced datasets. F1-Score can be represented as:

Confusion Matrices are diagrams that display the counts of predicted values versus the actual classes. Confusion Matrices are powerful as they allow you to notice where exactly in the model classification mistakes were made. For example if there is high number of False Positives, then there is an issue in the distinction between negative and positive values and possibly a pattern is being trained incorrectly. An example of a Confusion Matrix is visualized below:

Where in the diagram:

TP represents the True Positives (correctly predicted positive cases)

TN represents the True Negatives (correctly predicted negative cases)

FP represents the False Positives (incorrectly predicted positive cases)

FN represents the False Negatives (incorrectly predicted negative cases)

ROC/AUC curves are powerful tools that are able to graphically show the True Positive Rate agains the False Positive rate at various thresholds in the training process. The Area Under the Curve (AUC) helps quantify the results shown in the graph where an AUC=1 is a perfect classifier model and AUC=0.5 is no better than random guessing. This method is useful for imbalanced datasets. An example of a ROC/AUC graph is visualized below:

Graph 5: An ROC/AUC curve representing various classes overtime. ("Receiver Operating Characteristic", n.d.)

Logistic Regression Model Results

Our Logistic Regression Model achieved an accuracy of 0.85, meaning it correctly classified approximately 85% of the samples.

The Confusion Matrix provides a more detailed breakdown of the predictions. Out of all the actual positives, 306 were correctly identified and 18 were incorrectly predicted as positives. Among the negatives, 59 were correctly predicted, and 45 were missed. This means that the model was strong at identifying non-AIDs infected individuals, but struggled to differentiate actual AID cases. The Confusion Matrix is displayed below:

The precision for our negative class was 0.87 which was higher than the precision of our positive class which was only 0.77 in comparison. Additionally, the recall for the negative case was 0.94, much larger than the 0.57 for the positive case. The corresponding F1-Score was drastic as a result with 0.91 for the negative class and only 0.65 for the positive class. These reveal that while the model performs well overall, it is somewhat limited when predicting infection, prioritizing minimizing false positives over catching every infected case. A table of our results is shown below:

An ROC/AUC curve was plotted to further evaluate the model. The area under the curve for this model was 0.882 which signifies that the model can correctly differentiate between a randomly chosen infected and non-infected individual at an 88.2% probability, which is a relatively high score. Our ROC/AUC Graph can be visualized below:

Decision Tree Model Results

Our Decision Tree Model achieved an accuracy of 0.8178, meaning it correctly classified approximately 82% of the samples.

The Confusion Matrix provides a more detailed breakdown of the predictions. Out of all the actual positives, 283 were correctly identified and 41 were incorrectly predicted as positives. Among the negatives, 67 were correctly predicted, and 37 were missed. This means that the model generally struggled to differentiate both actual AID cases and non-AID cases due to its relatively higher missed values than the other models. The Confusion Matrix is displayed below:

The precision for our negative class was 0.88, which was higher than the precision of our positive class, which was only 0.62 in comparison. Additionally, the recall for the negative case was 0.87, slightly larger than the 0.64 for the positive case. The corresponding F1-Score followed a similar pattern, with 0.88 for the negative class and only 0.63 for the positive class. These reveal that while the model performs well overall, it is somewhat limited when predicting infection, prioritizing minimizing false positives over catching every infected case. A table of our results is shown below:

An ROC/AUC curve was plotted to further evaluate the model. The area under the curve for this model was 0.759 which signifies that the model can correctly differentiate between a randomly chosen infected and non-infected individual at a 75.9% probability, which is not the best score. Our ROC/AUC Graph can be visualized below:

Random Forest Model Results

Our Random Forest Model achieved an accuracy of 0.890, meaning it correctly classified approximately 89% of the samples.

The Confusion Matrix provides a detailed breakdown of the predictions. Out of all the actual positives, 306 were correctly identified, while 18 were missed. Among the negatives, 75 were correctly predicted, and 29 were incorrectly classified as positives. This indicates that the model performed well overall, particularly in identifying actual positive cases, though it still occasionally misclassified non-infected individuals. The Confusion Matrix is displayed below:

The precision for our negative class was 0.91, which was higher than the precision of our positive class at 0.81. Additionally, the recall for the negative class was 0.94, larger than the 0.72 recall for the positive class.

The corresponding F1-Score was 0.93 for the negative class and 0.76 for the positive class. These results show that the Random Forest model performs strongly overall, with a good balance between precision and recall. However, similar to the Decision Tree, it performs slightly better at identifying non-infected individuals than infected ones. A table of our results is shown below:

An ROC/AUC curve was plotted to further evaluate the model. The area under the curve (AUC) was 0.919, indicating that the model can correctly differentiate between a randomly chosen infected and non-infected individual with 91.9% probability, a very high metric. Our ROC/AUC Graph can be visualized below:

XGBoost Model Results

Our XGBoost Model achieved an accuracy of 0.886, meaning it correctly classified approximately 89% of the samples.

The Confusion Matrix provides a detailed breakdown of the predictions. Out of all the actual positives, 306 were correctly identified, while 18 were missed. Among the negatives, 73 were correctly predicted, and 31 were incorrectly classified as positives. This indicates that the model performed well overall, particularly in identifying actual positive cases, though it still occasionally misclassified non-infected individuals. The Confusion Matrix is displayed below:

The precision for our negative class was 0.91, which was slightly higher than the precision of our positive class at 0.80. Additionally, the recall for the negative case was 0.94, compared to 0.70 for the positive case. The corresponding F1-Score followed a similar pattern, with 0.93 for the negative class and 0.75 for the positive class. These results reveal that while the model performs very well overall, it is slightly limited when predicting infection, prioritizing minimizing false positives over capturing every positive case. A table of our results is shown below:

An ROC/AUC curve was plotted to further evaluate the model. The area under the curve for this model was 0.928, indicating that the model can correctly differentiate between a randomly chosen infected and non-infected individual with approximately 92.8% probability. The ROC/AUC Graph can be visualized below:

Hyper-parameter Tuned Model Results

The first model that we hyper-parameter tuned was our Logistic Regression model. Utilizing Grid Search, we were able to slightly improve and optimize the results of our model. This Logistic Regression model achieved an accuracy of 0.86, compared to the 0.85 accuracy of the original model. This demonstrates that tuning the regularization strength and penalty just very slightly enhanced the model's overall predictive performance.

The Confusion Matrix provides a detailed breakdown of slight improvements minimally as well. Compared to the original model, the tuned model shows a slight improvement in correctly identifying positive cases (63 vs. 59) and a minor reduction in misclassified negatives. Overall, both models perform well at detecting non-AIDS individuals, but tuning helped reduce false negatives for positive cases. These new results can be visualized below:

The precision, recall, and F1-score for each class also show subtle improvements. Precision for the negative class slightly increased from 0.87 to 0.88, while the positive class remained at 0.77. Recall for the positive class improved from 0.57 to 0.61, showing that the tuned model caught more actual positives. F1-score for positives increased from 0.65 to 0.68, indicating a more balanced performance between precision and recall after tuning. These new results can be visualized below:

However, for the ROC/AUC curve, there was a slight decline dropping from a 0.882 to 0.879. Although this value is still relatively high, this drop in ROC/AUC, the minor decrease suggests that tuning improved

classification of positive cases slightly at the expense of the model's overall ranking performance. It also overall signifies how tuning these hyper parameters did not effectively change the results or performance of the Logistic Regression model to make it significant.

The second model we hyper parameter tuned was our Random Forest model. This Random Forest model achieved an accuracy of 90%, slightly improving over the original accuracy of 0.89.

The Confusion Matrix shows the detailed breakdown of predictions. Out of all the actual positives, 75 were correctly identified and 29 were missed, while 310 negatives were correctly predicted and 14 were misclassified. Compared to the original model, the tuned version improved detection of negatives (slightly increasing from 306 to 310) while maintaining the same number of correctly predicted positives. The table below shows our new results:

Looking at the precision, recall, and F1-score, the tuned model improved the positive class precision increasing from 0.81 to 0.84, with recall remaining the same at 0.72. The F1-score increasing slightly from 0.76 to 0.78. For the negative class, precision remained high at 0.91, while recall increased from 0.94 to around 0.96, leading to a higher F1-score of 0.94. Overall, these metrics indicate that hyperparameter tuning slightly improved both overall accuracy and the model's ability to correctly classify negative cases, while marginally enhancing the positive class performance as well. A table of our new results are visualized below:

For the ROC/AUC curve, there was a very marginal improvement increasing from a 0.919 to 0.922. This slightest improvement shows that hyper-parameter tuning can help the model general data marginally better, but not enough to make too larger of a significant impact. Future work should be done over longer random searches to see if any drastic changes can impact the results of the ROC/AUC curve. A graph of our ROC/AUC curve for the new model can be visualized below:

The third model that we hyper-parameter tuned was our XGBoost model. The tuned model achieved an accuracy of 0.897, correctly classifying approximately 90% of the samples. This is a subtle increase from the untuned XGBoost model that has approximately a 88.5% accuracy percentage.

The Confusion Matrix provides a detailed breakdown of predictions. Out of all the actual positives, 306 were correctly identified, and 28 were misclassified. Among the negatives, 73 were correctly predicted, while 31 were misclassified.This demonstrates that the tuned model maintains strong performance in identifying non-infected individuals while improving detection of infected cases. The table below displays the Confusion Matrix of our tuned model:

The precision for the negative class increased slightly to 0.92, compared to 0.91 previously, while the positive class precision improved to 0.81 from 0.80. Similarly, the recall for the positive class increased to 0.75 (from 0.70), while the negative class remained at 0.94. Corresponding F1-scores were 0.93 for the negative class and 0.78 for the positive class, indicating a better balance between precision and recall for infected individuals. A summary of the classification metrics is shown below:

For the ROC/AUC curve, there was a very marginal improvement increasing from a 0.928 to 0.936. This slightest improvement shows that hyper-parameter tuning can help the model general data marginally better, but not enough to make too large of a significant impact. Future work should be done over longer random searches to see if any drastic changes can impact the results of the ROC/AUC curve. A graph of our ROC/AUC curve for the new model can be visualized below:

Conclusion Table

A conclusion table can be utilized to summarize our key findings. Our table is displayed below:

Future Work/Discussion

While current analysis and metric evaluation is able to detail out model performance, additional future steps could be used to strengthen findings and guide future model development. One potential way is by using statistical analysis tests to asses whether changes in performance metrics are statistically different. For example, if changing different values actually causes significant difference, or it just simply is due to randomness that values slightly increase or decrease. Using these tests can help guide future hyper-parameter tuning and adjustments to better optimize models

Another aspect of improvement would be hyper-parameter search. Within our research, due to computational limits, Random Search only ran for 10-20 iterations. To better improve this, future work can be conducted at higher iterations over broader sets of values for each hyper-parameter, better optimizing the search over a wider region. This can allow hyper-parameters to be better tuned overtime and likely come up with a better hyper-parameter tuned combination for each respective model.

Additional data gathering can be conducted as well. By collecting data within this field over various other variables or of just a broader demographic with more rows of data, this can improve model development and the patterns detected by models.

Conclusion

Across all models evaluated, the analysis reveals consistent patterns in predictive performance, with complex tree-based methods like Random Forest generally outperforming simpler models such as Logistic Regression and Decision Trees.

The Logistic Regression model achieved a solid accuracy of approximately 85%, demonstrating strong performance in identifying non-infected individuals but somewhat limited ability to detect positive cases, as evidenced by lower recall and F1-scores for the positive class. Hyperparameter tuning slightly improved the identification of infected cases, raising positive class recall from 0.57 to 0.61, though the ROC/AUC showed a minor decrease from 0.882 to 0.879, indicating that overall ranking performance was largely unchanged.

The Decision Tree model exhibited lower overall accuracy at around 82%, with both precision and recall for the positive class lagging behind other models. This reflects the model's limited ability to differentiate infected from non-infected individuals, highlighting the trade-off between model simplicity and classification performance.

More complex models such as Random Forest and XGBoost showed the strongest results. The untuned Random Forest achieved 89% accuracy and an ROC/AUC of 0.919, while XGBoost achieved 88.6% accuracy with an ROC/AUC of 0.928. Both models performed well in identifying non-infected individuals and showed reasonable detection of infected cases, though positive class recall was slightly lower than negative class recall. Hyperparameter tuning further enhanced these models: the tuned Random Forest reached 90% accuracy with an ROC/AUC of 0.922, and the tuned XGBoost reached 89.7% accuracy with an ROC/AUC of 0.936. Improvements were most notable in balancing precision and recall for the positive class, demonstrating that tuning can enhance detection of infected cases without sacrificing overall performance.

Overall, the results indicate that while simpler models like Logistic Regression and Decision Trees are useful for initial classification, more complex tree methods and boosted tree models like Random Forest Models and XGBoost perform at detecting AIDs better. ROC/AUC analysis across all models confirms that tuned Random Forest and XGBoost are most capable of distinguishing between infected and non-infected individuals, with probabilities exceeding 92%, making them the most reliable models for practical application.

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Evaluating Dance Therapy as a Non-Pharmacological Intervention for Adolescent Depression, Anxiety, and ADHD

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Abstract

Dance movement therapy (DMT) has emerged as a promising non-pharmacological intervention for improving emotional, psychological, and behavioral outcomes in children and adolescents living with depression, anxiety, and ADHD. This literature review synthesizes findings across multiple studies to discover the extent to which DMT is effective in reducing symptoms of depression, anxiety, and ADHD in children and adolescents. Overall, it was gathered that DMT is prevalent in reducing symptoms of these mood and behavioral disorders, promoting a better quality-of-life (QOL) in the short-term and longitudinally. Children and adolescents living with depression experienced enhanced emotional regulation and emotional expression through interpersonal connection and self-awareness, while children and adolescents living with anxiety experienced improvements in self-esteem, emotional regulation, social interaction, and self-attitude. Both groups also showed additional improvements in internalizing and somatic symptoms. Children and adolescents living with ADHD experienced improvements in motor regulation and behavioral control. Across all conditions, improvements were reflected in enhanced QOL and consistent reports of emotional and social progress from participants and caregivers. Although challenges remain regarding accessibility, instructor training, and long-term implementation, the reviewed studies collectively emphasize DMT's versatility, sustainability, and developmental relevance. Therefore, further recommendations remain for novel studies to expand and focus on examining DMT's long-term neurobiological outcomes and its potential integration into school-based mental health programs and digital telehealth platforms for added accessibility for rural areas.

Introduction

Dance movement therapy (DMT) can be defined as the psychotherapeutic use of moment to promote emotional, social, cognitive, and physical interaction of the individual (American Dance Therapy Association, 2020). Unlike traditional exercise or recreational dance, DMT emphasizes movement in a form of communication and emotional expression rather than performance or fitness. In a physical sense, dance therapy involves three distinct phases: warm-up, dance practices, and relaxation or cool-down. Each phase is critical to a well-rounded experience. The focus of these phases lies in cultivating self-awareness, regulation, interpersonal connection, and reflection through guided movement. For this review, "dance therapy" encompasses structured, movement-based interventions designed with therapeutic intent, including programs that integrate improvisation, choreography, and facilitated group interaction to promote psychological well-being in children and/or adolescents.

There is a need for novel approaches to therapy specifically in the youth and adolescent population (age 4-18). Youths have an increased focus due to their point of growing neurodevelopment, a need for support in peer dynamics, and a heightened risk for internalizing and externalizing symptoms due to their early age. Adolescence represents a particularly critical developmental period, which can be defined as a time during early postnatal life when the development and maturation of functional properties of the brain experience heightened plasticity, which is strongly dependent on experience or environmental influences (Gariépy et al., 2020). This suggests that experiences during this period can have lasting effects on neural pathways related to emotion, behavior, and self-control. Because dance therapy engages multiple domains, including motor

coordination, sensory integration, emotional expression, and social interaction, it aligns naturally with the needs of this age group. This impact cannot similarly be seen later in life in adults, where they would be past their critical period for development and the same influence would often not yield as much improvement in results of dance therapy. Although studying youth populations presents unique challenges, such as the need for parental consent and inconsistent adherence to intervention, these limitations do not diminish the importance of early intervention. Addressing emotional dysregulation during formative years can shape lifelong trajectories of mental health and resilience while continuing to further the field of non-pharmacological intervention.

The growing demand for non-pharmacological interventions in pediatric mental health also makes DMT particularly relevant. Traditional pharmacological solutions for mental health improvements are limited in terms of accessibility, specifically in cases of low-income households where constant prescriptions are simply not a realistic venture. Long-term sustainability also presents with difficulty, where parents are simply losing interest in heavily medicating their children and youth have significant trouble when it comes to consistently taking medication in the first place. DMT, on the other hand, provides a new avenue for both accessibility and sustainability when treating mental health. The only requirements for DMT are attendance and participation, without any prior experience, eliminating a cost factor negatively impacting pharmacology. It is also more reliable in terms of consistency with it being an approach that is more appealing to youth than taking medication.

Mental health disorders, specifically depression, anxiety, and ADHD, are mood and/or developmental disorders which can be improved with the help of dance therapy. Depression is defined as a mood disorder that causes a persistent feeling of sadness and loss of interest that interferes with daily functioning. Depression affects 23 million children and adolescents globally, and 9% of these children ages 3-17 are living with a diagnosis of major depressive disorder (Mayo Clinic, 2022). Anxiety is defined as a persistent worry and fear towards everyday situations, often characterized by panic attacks, physiological arousal, and interference with daily living, often categorized into generalized anxiety and panic disorder. (Mayo Clinic, 2025). Anxiety disorders affect approximately 21% of children ages 3-17 who are living with a current diagnosis (CDC, 2025). ADHD is defined as developmental, attention-hyperactivity/deficit disorder constituting one or more of the following: inattention, hyperactivity, and impulsivity (NIH, 2024). In the United States, seven million (11.9%) of children ages 3-17 are living with a diagnosis of ADHD.

Many adolescents with depression or anxiety experience somatic symptoms, which are physical manifestations of psychological distress such as fatigue, headaches, and muscle tension (Merced et al., 2022). These experiences frequently overlap with internalizing problems, which describe the inward expression of distress though depression, anxiety, or psychosomatic complaints. Most individuals who experience internalizing problems developed them in early childhood, most commonly in middle childhood to adolescence. In fact, 7% of children living with anxiety and 3% of children living with depression have been recorded to demonstrate internalizing symptoms (Bajaras-Gonzales et al., 2020), DMT directly addresses these symptoms by improving emotional regulation, self-awareness, and adding an element of interpersonal connection through movement. These improvements correspond with increased quality of life (QOL) in this population, which encompasses an individual's perceived well-being. Enhancing QOL in adolescence not only alleviates immediate distress but also improves long-term mental health outcomes.

The widespread increasing presence of these mood disorders stresses the critical need for intervention, more specifically one sustainable for long-term benefits that improve quality of life for children and adolescents longitudinally. Fortunately, the integration of movement, emotional expression, and social connection positions dance therapy as a promising intervention for adolescent mood disorders. Therefore, this review seeks to evaluate the extent to which DMT is effective in reducing symptoms of depression, anxiety, and ADHD among children and adolescents. Understanding the impact of DMT during childhood and adolescence is essential for expanding evidence-based, creative, and accessible approaches to youth mental health. Additionally, it addresses the need for improvement and innovation in non-pharmacological treatment

of these disorders to promote continual enhancement within longitudinal quality of life measures for youth living with a diagnosis of depression, anxiety, and/or ADHD.

Dance Therapy as an Intervention for Depression and Emotional Well-Being

When looking for substantial growth when it comes to benefits of DMT on symptoms of depression, it is critical to see improvements in somatic symptoms and internalizing problems. These symptoms often manifest physically as headaches, stomachaches, fatigue, muscle tension, and sadness, which are direct reflections of the emotional distress experienced by individuals living with depression. DMT works towards these improvements by using the interconnection of developments in motor coordination, sensory integration, and other physical aspects with emotional regulation further relating to well-being.

Jeong et al. (2012) studied a population of 51 middle school senior girls (mean age:16) with a pre-confirmed diagnosis of depression to investigate psychological health and changes in neurohormones of adolescents with mild depression after 12 weeks of DMT. The therapy consisted of a 45-minute dance class three times a week, focused around four major themes: a) awareness of the body, the room, and the group; b) movement expression and symbolic quality of movement; c) movement, feelings, images, and works; and d) differentiation and integration of feelings. These themes aimed to foster mindfulness, self-expression, and positive internal attitudes through synchronized movement. Following DMT, participants underwent "brainbow" and the results demonstrated significant neurohormone improvements, including a decrease in cortisol concentration, an increase in serotonin concentration, and a decrease in dopamine concentration. These changes not only provide significant biological data on the effect of DMT on depression but also demonstrate improvement in emotional regulation within a scientific context as opposed to solely looking at external signs. Additionally, the findings emphasize DMT's ability to promote neurochemical balance and regulate stress responses that counteract depressive symptoms, reinforcing the role of dance as a somatic outlet for emotional processing. The findings emphasize the improvement in behavioral symptoms, such as somatization and interpersonal sensitivity, which were presented as a result of the changes in the neurochemical balances. This stressed the outcomes beyond that of simply a biological shift, instead connecting the real presenting behavioral benefits provided as the outcome of that neurohormone change.

In another study by Duberg et al. (2013), self-rated health (SRH) of adolescent girls with internalizing problems was investigated in relation to dance intervention's influence. The study consisted of 112 girls ages 15-18 who had recent visits to their school nurse for somatic symptoms without the necessity of a diagnosis of depression. This inclusion criterion allowed researchers to target a broader population of adolescents who may not yet meet clinical thresholds but still experience emotional distress, making the study's findings more generalizable to early intervention models. The DMT was a dance intervention trial with follow-ups post study at months 8, 12, and 20. Sessions took place twice a week for 8 months lasting 75 minutes including 15 minutes of warm-up, 40 minutes of dance practice, 15 minutes of stretching, light massage in pairs, and relaxation, and 5 minutes of reflection. Each session had a style of focus, including jazz, contemporary dance, and African dance with the guidance of one of three designated instructors. When looking at the combination of experience in the program and patient adherence, the study demonstrated improvements of self-rated health measured using a questionnaire containing 88 questions regarding lifestyle, self-rated health, emotional distress, psychosomatic symptoms, feelings, depression, sleep, school, interests, friends, leisure time, and how they enjoy dance taken by the participants, along with improved somatic symptoms, each critical to progress in adolescents with depression including internalizing problems. This combination of adherence and experience contributed to participant's improvements in social connection, self-esteem, and overall positivity, which are essential for longitudinal emotional resilience in adolescents.

Huang et al. (2023) also investigated the effect of dance interventions on somatic symptoms and psychological health of teenage girls with the additional goal of also providing scientific guidance to schools

on mental health. The study included 100 young girls ages 10-18 with the criteria including internalizing difficulties relating to stress and multiple visits to their school nurse for somatic symptoms. The DMT consisted of dance classes twice a week for one year, with the duration of each class being one hour and ten minutes: 20 minutes dedicated to warm-up, 45 minutes dedicated to dance practices on body awareness, and 5 minutes dedicated to introspection or otherwise identified as reflection. Each class included choreographed dances with styles of African dance, show jazz, and street dance, with room for improvisation with the aim of a pleasant experience free of judgement or competition between participants. The study resulted in a strong positive relationship between somatic symptoms and emotional distress. Additionally, results showed improvements in somatic symptoms and emotional distress, proving beneficial results of emotional regulation. This study also provided merits for long-term DMT for continued benefits. The longitudinal design allowed researchers to observe the sustained impact of DMT, supporting that consistent engagement, rather than short-term participation, proved the key for lasting psychological improvements in adolescents.

Overall, the results discussed above provide evidence that DMT has a niche promoting emotional regulation within adolescents experiencing symptoms of depression. These outcomes directly correlate to positive developments in QOL in the short-term and longitudinally. Additionally, this evidence suggests that the interconnect of self-awareness and interpersonal connection leads to improvements in emotional expression, which can be continually seen in the daily life of the adolescents who participated in these studies and hopefully can be sustained by continued dance therapy for long-term success. This impact is the most significant for children and/or adolescents living with depression because it leads to a more optimistic future and improved somatic and internalizing symptom management.

Dance Therapy as an Intervention for Anxiety and Self-Determination Theory

Dance movement therapy (DMT) acts as an avenue for success in improving symptoms of anxiety, and this is largely due to the impact of the self-determination theory. The self-determination theory (SDT) discusses the factors that encourage or hinder human motivation, including both internal and external factors of influence (American Psychological Association, 2025). SDT addresses three basic psychological needs contributing to human motivation: autonomy, competence, and relatedness. When these needs are met, individuals experience greater emotional stability (ig. self-confidence, self-esteem) and improvements in overall psychological well-being. In the context of DMT, these elements are fostered through creative expression, freedom of movement, and supportive social interaction. By promoting autonomy in self-expression, competence through skill development, and relatedness through connection with others, DMT addresses the emotional and social barriers that commonly underlie anxiety in children and adolescents.

In a pilot study conducted by Dunberg et al. (2016), researchers investigated the experiences of adolescent girls participating in a dance intervention for the treatment of internalizing problems. The study included 112 girls ages 13-18 (mean age: 16) demonstrating internalizing problems and making frequent visits to the school nurse for somatic symptoms. The DMT intervention consisted of two 75-minute dance classes weekly over 8 months. Each class dedicated 15 minutes to warm-up, 40 minutes for dance practice emphasizing body awareness, 15 minutes for stretching, paired massage, and relaxation, and 5 minutes for reflection. Various dance styles were incorporated into choreographed sections of the class, including African, show jazz, and street dance, while allowing room for improvisation to encourage a non-competitive and supportive environment. Findings suggested that DMT gave participants access to and enriched personal resources such as self-trust via direct feedback, emotional expression, and social connectedness. Participants described feelings of togetherness, enjoyment, trust in their abilities, and freedom of movement, which collectively enriched their sense of competence and belonging. This overarching narrative was encouraging, considering that it suggested improvements in expression of internalizing problems and reduction of somatic symptoms. These results also emphasize a deeper transformation which occurs when emotional safety meets bodily expression, along with a recurrent theme of improved self-esteem, driving a lasting motivation to seek self-care and engagement that

continues beyond the context of DMT.

The role of DMT in addressing anxiety is further supported by a similar study by Li et al. (2025), who examined the effect of dance intervention on left-behind children (LBC). LBC can be defined as youth who suffer from social anxiety and low self-concept, due to limited parental presence. The study included 402 Chinese students (years one through seven) who scored eight or higher on a social anxiety scale and below the 30th percentile for self-concept. The structured DMT intervention consisted of four weekly 45-minute sessions, each divided into a 5-minute warm-up, 35-minute dance training, and 5-minute relaxation. The warm-up emphasizes exercises moving the legs, wrists, head, neck, shoulders, waist, and hips. The dance training centered on the Latin dance style of Cha Cha Cha, taught in three phases: basic Cha Cha Cha movements, solo Cha Cha Cha combinations, and partner combinations. Relaxation included breathing exercises and stretches targeting movements of the shoulders, neck, arms, legs, and back. The findings of the study revealed significant reductions in social anxiety scores and improvement in self-concept across all groups. More specifically, participants reported decreased fear of negative evaluation, social avoidance distress, and social anxiety, along with higher ratings in happiness, popularity, and life satisfaction.

Figure 2. Self-concept scores of popularity, happiness and life satisfaction in left-behind children (LBC) before and after dance movement therapy by Li, X., Yang, Q., Zhou, Z., Zeng, M., Lu, C., & Dong, W. (2025). Effects of a 12-week dance intervention on left-behind children with co-occurring social anxiety and low self-concept. Frontiers in Psychology, 16, 1491743. https://doi.org/10.3389/fpsyg.2025.1491743.

These findings suggest that DMT provides a structured environment where LBC children can experience competence through skill mastery and relatedness through partner interaction which may be absent in their homes. Additionally, Li et al.'s findings illustrate how DMT builds social and emotional resilience in children experiencing unstable home lives by integrating motor learning with interpersonal connection. As participants developed both physical coordination and relational trust, they simultaneously strengthened neural networks that support self-esteem and adaptive coping, demonstrating how psychosocial growth and neural plasticity converge to alleviate anxiety.

Similarly, in a follow-up study by Dunberg et al. (2020), the reduction of anxiety-related somatic symptoms and emotional distress was investigated using a structured dance intervention. The study consisted of 112 girls ages 13-18 (mean age: 16) with repeated visits to the school nurse for somatic symptoms and anxiety-related internalizing problems. Because this study was conducted as a follow-up to the previous Dunberg et al., (2016) study, it utilized the same DMT curriculum. This study was uniquely separated from the pilot study in that it assessed quantitative over qualitative data using five-item scales. The study demonstrated a significant reduction in emotional distress and somatic symptoms, showing improvement not only in emotional regulation but also in self-perception and confidence. Furthermore, twelve months following the end of the DMT program, participants continued to report these improvements, and symptoms did not begin to return to baseline until the eighteen-month mark. These outcomes align with SDT's central tenets by demonstrating enhanced autonomy (freedom of movement), competence (mastery of physical and emotional skills), and relatedness (connection with peers). Neurobiologically, these improvements may result from regulated cortisol secretion and improved sensorimotor integration, as participants re-engaged bodily awareness with emotional processing through coordinated movement. The enduring improvements reported emphasize the importance of consistency in participation and suggest that DMT may serve as sustainable, non-pharmacological treatments for anxiety across longitudinal developmental stages.

In summary, the results of these studies provide strong evidence of DMT's role in promoting self-esteem, emotional regulation, and positive self-attitude among children and adolescents living with anxiety. These outcomes directly contribute to improved quality of life (QOL) in both the short-term and longitudinally. Moreover, they emphasize DMT's potential as a non-pharmacological approach to managing anxiety through routine psychosocial and neurobiological mechanisms. By fostering autonomy, competence, and relatedness,

DMT not only reduces somatic and emotional symptoms, but provides opportunity for youth to consistently modulate their emotional expression and seek rewarding social interaction. Because of this, DMT represents a uniquely integrative intervention which offers a pathway for individuals to regain control over their bodies and minds, reconnect with their emotions, and re-establish motivation and purpose. Through this continuous feedback between movement, emotion, and self-regulation, DMT provides both immediate relief and enduring psychological growth, reaffirming its value as a sustainable therapeutic model for youth living with anxiety.

Dance Therapy as an Intervention for ADHD and Behavioral Regulation

Dance movement therapy (DMT) incorporates a new aspect of developmental encouragement in children and adolescents with ADHD. ADHD's categorization as a neurodevelopmental disorder is separated from mood disorders in that they are even more persistent in childhood and impact cognitive and emotional development, having a significant contribution on children and adolescents continuing into adulthood. Motor regulation is often directly impacted in neurodevelopmental disorders, becoming a common and observable symptom, and DMT has a unique approach to supporting the motor regulation pathway through structured movement and rhythmic coordination. Engagement with DMT can also lead to a positive effect on behavioral regulation over time due to activation of the cerebellum and cerebral cortex, which are responsible for attention, coordination, behavioral control, creation of a motor plan, and repeated motor learning. These benefits are particularly important for long-term success, contributing to growth in daily functioning that can positively influence children and adolescents as they age. Therefore, DMT is established not only as a therapeutic outlet for emotional release but also a developmental intervention that aligns physical coordination with behavioral stability. By strengthening and upregulating motor pathways that underlie attention and impulse control, DMT assists children in building foundational skills for lifelong motor regulation.

A study by Anderson et al. (2024) investigated the feasibility and impact of dance DMT on children and adolescents with neurodevelopmental disabilities (NDD), including ADHD. The study consisted of 43 male and female children and adolescents ages 4-17 with diagnosed NDD. The DMT consisted of weekly one-hour classes on Zoom, specified as either a group class or "buddy" class. Group classes start with stretches, leading into "across the floor" exercises frequently incorporating ballet techniques followed by a short break. After the break, participants work on a short choreography segment, then a dance-based game and time with their "buddies," or otherwise identified as trained professionals, where they work on a specific task before sharing with the class. "Buddy" classes were structured similarly but instead began with buddy time and ended with the same cool-down. The findings demonstrated significant improvements in motor skills within an unpredictable, dynamic environment. This impact is substantial in showing how DMT can lead to motor regulation, social communication, and motivational improvement, which contributes directly to behavioral outcomes in the children and adolescents.

The inclusion of "buddy sessions" created opportunities for structured social engagement, reinforcing both attention and emotional connection. These results directly demonstrate the adaptability and accessibility of DMT, even through virtual delivery, showing that interactive movement fosters regulation across cognitive, motor, and social domains. The improvements in social motivation and engagement suggest that movement-based therapies, such as DMT, may stimulate executive functioning and mirror neural development patterns, promoting consistent motor behavioral learning and gains over time.

In a similar study by Shilpa et al. (2015), researchers aimed to discover the effectiveness of DMT among children living with ADHD. The study included children ages 6-9 who were assessed by teachers and parents as displaying symptoms of ADHD. The DMT consisted of specific child aerobic exercises with the incorporation of selected songs on a CD designated for each exercise, organized into three phases: warm-up, moderate intensity movement, and cool-down. Warm-up included moves such as step-touch, marching, and double-touch with hand moves and crisscross steps. Moderate-intensity movements included cheer-up leader

routines, internal and external hip rotations, and salsa steps with hand motions, all repeated at a minimum of 16 times with rests. The cool-down included forward and backward stretches and shoulder rotation. The findings showed significant differences on the SNAP-IV rating scale of ADHD symptoms before and after the intervention, indicating improvements in behaviors such as attention, motor regulation, and aggression. As reported by participants' parents, there were also improvements in social skills, confidence, patience, and activity daily performance, such as engagement with peers, a strong indicator for recommendation of this therapy. Over time, these improvements in social interaction and self-regulation express the benefit dance therapy provides to daily functions that are imperative to behavioral improvements and especially critical for quality of life in ADHD populations.

These results were similarly seen in a study by Grönlund et al. (2012) investigating the effect and value of DMT as an alternative treatment for aggressive and destructive young boys showing symptoms related to ADHD. The study consisted of 5-7-year-old boys of average intelligence meeting the DSM-IV criteria for ADHD and presenting with aggressive and/or destructive behavioral tendencies. The DMT consisted of ten 40-minute sessions weekly for three months, incorporating a quick warm-up, followed by a restful break, a small massage by the therapists to make the boys more relaxed, and ending with teaching short dances independently. The case studies demonstrated particular improvement in socio-emotional behavior in one of the boys, including better self-control and reduced aggression. Both boys received benefits to their motor skills. These gains also led to improvements in social skills and decreased expressions of anxiety, clearly showing the impact of DMT on outcomes not only improving motor regulation, but also transforming maladaptive behaviors into cooperative, self-regulated responses that sustain behavioral regulation when practiced consistently.

This study also looked more closely at two individual case studies within the program. The first observed the impact on a boy by the name of "Tom." Tom showed significant improvement in balance, sleep, social interactions with both peers and adults, and overall anxiety. He was less frequently aggressive, and specifically less prone to outbursts. Tom's mother indicated the significance of these outcomes, addressing her astonishment in Tom's immediate compliance with the dance therapists without her present. She also discussed how relaxed Tom was after dance therapy sessions in a way that was not possible before. She emphasized wanting more DMT for her son due to the profound benefits it provided her son. The second case study was based on a boy by the name of "Peter." After the intervention, Peter had improved social skills, seen in his less controlling behavior, reduced stress and anxiety, and fewer outbursts due to his increased control and awareness of his own emotions. He was less aggressive and hyperactive, with visible improvements in concentration. Both Peter and his mother wanted to continue with DMT after the study ended as well, reinforcing how meaningful the therapy had become in their daily lives. These case studies provide a deeply human dimension to DMT's therapeutic success, showcasing that behind every improvement in behavior is an equally important gain in therapeutic alliance and adherence. The willingness of both children and parents to continue beyond the study period reflects not just short-term success, but the establishment of consistency and motivation that support long-term well-being.

Overall, the studies' findings show strong evidence of the DMT's impact on the motor regulation and behavioral outcomes of children and adolescents with ADHD. These results are significant indicators of the benefits DMT has on quality of life (QOL), particularly seen in translation to longitudinal benefits with continued, consistent therapy. DMT has unparalleled influence on the motor regulation pathway, which is demonstrated through frequent results of improvement in motor skills and behavioral control, suggesting a neurobehavioral link between movement synchronization, executive function, and motor learning. These results also emphasize the potential for long-term benefits with consistent participation, demonstrating how structured DMT and creative movement can promote sustained attention, social integration, and behavioral-motor regulation. Over time, this transformation supports not only ADHD-related symptom reduction but also growth in independence and resilience, contributing to a stronger foundation for lifelong

behavioral and emotional regulation.

Discussion

The impact of dance movement therapy (DMT) is prevalent in reducing the symptoms of depression, anxiety, and ADHD in children and adolescents. DMT promotes emotional regulation in children living with depression, along with sustaining emotional expression through interpersonal connection and self-awareness. Children and adolescents living with anxiety received improvements in self-esteem, emotional regulation, and self-attitude due to DMT, all attributed to dance therapy's fostering of autonomy, competence, and relatedness as described by Self-Determination Theory. These components gave children and adolescents greater ability to modulate emotional expression and engage confidently in social interaction. Additionally, children living with anxiety and/or depression consistently showed improvements in internalizing symptoms, showing positive developments in emotional distress and somatic complaints, reflecting DMT's direct influence on both physiological and emotional well-being.. In children and adolescents living with ADHD, motor regulation and behavioral improvements were particularly evident in response to DMT, suggesting a neurobehavioral link to movement synchronization, executive function, and motor learning. These individualized improvements, as highlighted in the case studies, emphasize DMT's impact on the well-being of ADHD children and adolescents, seen in parental enthusiasm for continued sessions and the independence and resilience demonstrated by the children. Collectively, the results across all three conditions consistently demonstrate DMT's capacity to enhance quality-of -life and the continued success of children and adolescents, emphasizing advancements short-term and longitudinally. Therefore, DMT serves as an effective holistic intervention, one that strengthens emotional, cognitive, and behavioral domains simultaneously, creating a foundation for lifelong adaptability and resilience.

Much of the evidence also implied the need or recommendation for longitudinal application of DMT due to the positive responses from participants and the need to derive long-term benefits. Many studies observed that long-term participation of children and adolescents led to greater and more stable improvements in QOL, emphasizing that the therapy's effects are cumulative rather than immediate. DMT's consistency over time appeared to facilitate neuroplastic changes in emotional regulation pathways, reinforcing both self-control and coping mechanisms. However, implementing DMT on a larger scale comes with logistical challenges, particularly with integrating similar programs into school or healthcare environments. One of the most pressing issues related to accessibility is location; DMT is dependent on a centralized space, which limits access for children in rural or under-resourced areas who lack a community willing to invest in this type of program. Additionally, DMT also requires the availability of thoroughly trained instructors, and specialized dance styles may require teachers with expertise in multiple movement forms. For example, many hip-hop teachers are not educated or trained in the ballet art form. Another critical aspect of DMT is maintenance and structure. DMT is successful because of consistency and a very precise design with frequent visits, often multiple times in one week, leading to QOL enhancement unparalleled with inconsistent, relaxed formats. This consistency contributes to improvements in mood, self-esteem, and symptoms of depression, anxiety, or ADHD in participating children all of which directly contributed to a better QOL. While accessibility and implementation challenges exist, they are outweighed by the proven benefits of consistent and structured DMT programs. The evidence supports that with proper integration and training, DMT can serve as an equitable, sustainable mental health intervention for youth.

While DMT can face barriers to successful implementation, it is most often a reliable, sustainable, and worthwhile therapy type allowing for easy access to improvements in some of the most difficult aspects of childhood and adolescence. One of DMT's most unique characteristics is improvisation, which creates an environment facilitating creative expression and encouraging self-esteem and non-competition. Improvisation is a special experience and can rarely be found in any other form of exercise, likely contributing to the positive outcomes in the therapy form. DMT is also accessible in that it does not require many, if any materials and can

be practiced anywhere there is open space. Furthermore, DMT does not require any previous dance experience, making it approachable and inclusive, an additional factor in the ease of accessibility. Although dance instruction is often associated with high cost, DMT programs can be sustainable when integrated into clubs or extracurriculars, making them more sustainable and easier to maintain long-term. Integrating DMT into familiar community settings also allows for natural referral pathways through school counselors, mentors, or healthcare providers, especially when paired with established therapeutic models like cognitive-behavioral therapy. Under these circumstances, it also becomes easier for children to receive a referral when they demonstrate a true need for intervention. In many cases, DMT can serve as a viable alternative or adjunct to medication in children and adolescents living with mood disorders, especially for children who struggle with compliance, side-effects, or whose parents seek non-pharmacological options. Instead, DMT creates a different avenue for interventional treatment before taking medication. Overall, DMT's unique blend of creative expression, accessibility, and therapeutic depth makes it a transformative form of care. It offers children an opportunity to self-regulate and express emotions safely, while also empowering caregivers and educators with an approachable, community-based intervention.

DMT is best applied when sessions occur frequently and over longer periods of time. More specifically, studies most often showed success with two sessions weekly spaced out across eight to twelve months, a routine that allows emotional, social, and motor progress to consolidate. DMT appeals most greatly to the child and adolescent demographic due to its connection to motor coordination, sensory integration, and emotional regulation, which work together to strengthen neural pathways influencing emotion, attention, and behavior. Because the child and adolescent years represent a critical period for neurodevelopment and identity formation, the effects of DMT during the ages of four to 17 are especially enduring. DMT's success is also unspecific to any type or style of dance; evident improvements were seen across a range of movement forms, suggesting that the therapeutic element lies in movement expression itself, rather than any specific technique. Future studies should focus on examining DMT's long-term neurobiological outcomes, such as its influence on activation of the prefrontal cortex, cerebellum, and corticolimbic networks, as well as its potential integration into school-based mental health curriculums and digital telehealth platforms for rural accessibility. Most importantly, DMT changes the treatment landscape for children experiencing mood and developmental disorders, opening greater possibilities for their futures than were previously imagined. It not only improves clinical symptoms but also empowers children living with depression, anxiety, and ADHD to reconnect with joy, creativity, and self-worth, all key ingredients for lifelong mental wellness and resilience.

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