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Physical Activity Levels as Effectors on Brain Evolution

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Abstract

As modernization and sedentary lifestyles are significantly increasing, the direction in which neurological evolution takes place may be startling. The development of a larger brain allowed humans to differentiate from primates with our more complex brain development and cognition. This transition has previously been solely quantified through the nucleotide sequence alterations and behavioral analysis. However, the discovery of epigenetics gives rise to the contribution of environmental factors on heritable ways through which our DNA is expressed. The methods by which methylation and histone modification are inherited are varied, and few studies have been published analyzing the transgenerational effect of exercise. Transgenerational inheritance (F0-F2 offspring) requires differentiation from intergenerational effects (F0-F1 offspring), removing the contribution of the environmental stimuli altering DNA via embryologic exposure. Few groups have investigated the transgenerational contributions of parental exercise. This review will briefly discuss the molecular mediators which can measure evolutionary development of the brain and how the sudden transition to modernization and sedentary lifestyles could affect the future evolution of our brain.

Background in Metabolism and Brain Evolution

Current studies of human evolution describe the differentiation of primates to *Homo sapiens* through human metabolic rate. Researcher efforts have targeted metabolic utilization as the key differentiating factor between humans and primates. Isotypes of hydrogen and oxygen were fed to a variety of primate species and humans to measure the total calories burned daily (TEE). The basal metabolic rate and rates of total energy expenditure (TEE) were significantly higher in human than even their closest relative, the chimpanzee, after adjustment for stored body fat [1]. Species variation can be difficult to demonstrate exact causation in the differences of metabolism

along the evolutionary timeline. The factor that is evident in the development of larger human brains is the increased basal metabolic rate, indicating that organs are able to efficiently metabolize energy at resting state.

The TEE of a species is impacted by the work performed in a day, as the total calories burned would be significantly altered by introduction or withdrawal of physical activity. Modernization has depleted the need for physical activity by removing the previously energy-demanding daily activities such as water consumption and food-seeking behaviors. Hunter-gatherer communities often reach eight to nine hours of physical activity in their pursuit of shelter and nutritional necessities [2]. Urbanization has created the ability to obtain these requirements while maintaining a sedentary lifestyle. The physical activity and increased metabolic rate which has evolved our brain to reaching a heightened potential may be at risk with our revolutionized lifestyle.

This metabolic approach of comparison in energy usage studies to understand the creation of a large and efficient brain does not adequately account for all factors. The correlation is evident; however, the causation is unclear. This review aims to discuss molecular mediators which may be directly impacting how the brain utilizes energy to allow humans increased cognitive function and brain plasticity. The connection between physical activity and mediators such as IGF-1, BDNF, and miRNAs demonstrates causation of this ‘better brain’ at the molecular level. These mediators have been shown to have an inheritance effect on expression at the individual level and for generations to come. New scientific studies linking epigenomics to physical activity may reveal the secret to our previous success in climbing the evolutionary ladder. Techniques in monitoring the epigenetic effect of exercise allow researchers the ability to focus on specific modulators in defining the causative role of exercise.

Introduction into Epigenomics

Selective activation and repression were crucial biochemical processes which allowed our higher species immense abilities under gene regulation. Exploration into the field of epigenetics has allowed researchers to understand the underlying mechanisms of phenotypic projections. This developing field will allow a further explanation in differentiating between correlation and causation regarding the environmental impact on the expression of our genetic code. Previously, it had been recommended to maintain a healthy lifestyle in passing down those 'genes' to future generations. This statement warrants the scientific explanation of how such mechanism would truly take place. Through chromatin immunoprecipitation and methylation profiling, unique histone modifications and DNA methylation at promoter regions could be analyzed under experimental conditions in support of determining causation. The generational heritability of slight genomic modifications has been found to demonstrate the possibility of passing down our adaptations to environmental stimulants. Understanding the methylation of specific promoter sites can give a causative role between the stimuli and effected DNA.

The DNA sequence and proteins have an intimate relationship in the condensation of the genetic code. There are two major ways in which the expression of genes can be controlled: DNA methylation and histone modification [3]. Histones are instrumental in the modeling of chromatin, and by modifying the N-terminal tails of these proteins with acetyl or methyl groups, the expression of genes can be affected. Methylation of DNA at CpG nucleotides creates the opportunity for activation or repression. CpG dinucleotides represent a small portion of the genome; however, when found, they are located in clusters known as CpG islands [4]. There is a strong association between these islands and genes which are expressed. Global methylation has been focused on the quantification of these CpG islands. There has been direct correlation

between methylation and pathogenesis of disease, which creates demand for further specificity in the role of methylation and contributing factors.

Global Methylation

DNA methylation, compared to histone modification, has been found to be more significant in epigenomics through quantifying the number of methylated regions on DNA. Global methylation has been studied in a variety of publications to have an impact of pathogenesis of disease. However, the terminology of global methylation does not specifically quantify how researchers have measured such methylation. There are three categories in which global methylation can be evaluated: genome-wide, repetitive elements, and multiple gene regions. Global methylation has been recommended to be quantified via LINE-1, long interspersed nuclear elements, which are repetitive DNA retrotransposons duplicating throughout DNA [3].

LINE-1 has shown contrasting results when measuring for global methylation correlated to exercise. When utilizing bisulfite-converted DNA and PCR via blood samples, there was increased global methylation following increased physical activity [4]. The physical activity in this study was not equal among participants as an accelerometer was worn by participants to measure activity level. Average daily time spent in moderate exercise for participants was 17.5 minutes. This study provided promising hope as it demonstrated significantly higher methylation rates in participants who engaged in scheduled physical activity. However, once variable factors such as ethnicity, body mass index, and smoking status were adjusted for association, there was no longer a statistic significance.

When quantifying global methylation through a genome-wide analysis, contrasting results were found. Adult male Wistar rats were split into two experimental groups: exercised and sedentary. The exercised rats ran on a treadmill during a twenty-minute period, for five days during the

week, over a 22-day experiment. Hippocampus samples of the offspring were removed at post-natal day 53, with DNA isolation occurring immediately. The MethylFlash Methylated DNA 5-mC Quantification Kit was utilized in measuring genome-wide methylation compared to LINE-1 evaluation. There was significantly decreased genome-wide global methylation in the exercised group [5]. The varied findings under different disciplines of quantifying global methylation give reason that this method has not yet demonstrated consistent findings in the protective mechanisms of physical activity.

Brain-Derived Neurotrophic Factor

Brain-derived neurotrophic factor, encoded by the BDNF gene, is a protein which contributes to neuronal excitability and learning/memory function. BDNF demonstrates an epigenetic mechanism by allowing post-transcriptional modifications to DNA and nuclear proteins to modulate its regulation. There are multiple promoter sites of this gene; however, Promoter IV has invoked majority of research interest. Transcription of this promoter is suppressed by methyl-CpG-binding protein (MeCP2), which creates a repressor complex and silencing effect. Neuronal depolarization causes MeCP2 to become phosphorylated and lift from the promoter to allow gene expression [6]. From the behavioral standpoint, depressive behavior in rats causes methylation of histone H3 and suppresses Promoter IV of BDNF. Some individuals have unmethylated BDNF promoter sites, which could cause variability in results.

Exercise has shown to have a positive impact on the expression of BDNF and hippocampal synaptic plasticity through two primary mechanisms. Calcium/calmodulin-dependent protein kinase II (CaMKII) and cAMP response element binding protein (CREB) are cascades involved in methylation-related expression of BDNF [7]. Exercise modulating the expression of brain-derived neurotrophic factor could have significant clinical implications as the protein is

responsible for a variety of cognitive abilities. After one week of exercise in the rat hippocampal model, there was significantly reduced methylation of the *Bdnf* exon IV promoter region involving 230 base pairs. CpG site –148 bp was most impacted by exercise, demonstrating significant demethylation compared to sedentary control models. Overall methylation in this CpG site was reduced from 59.2% methylation in sedentary rats to 18.4% methylation in active rats. The acetylation of histone H3 and H4 (as a control) was also ChIP quantified under exercise as a modulator. There were no significant changes to H4, while H3 acetylation in the BDNF promoter IV region is enhanced under voluntary exercise. Through Western Blot analysis, phosphor-CREB/CREB activity was heightened by 53% in the exercise experimental group compared to sedentary group. These findings support the role of exercise in the beneficial effects of recruiting protective mechanisms for cognitive ability.

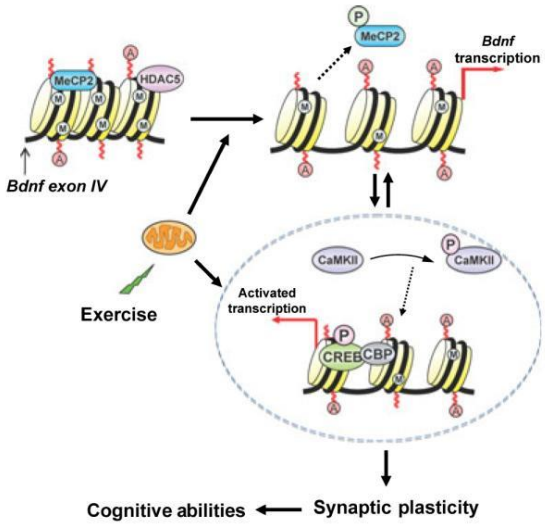

Fig. 1 [7]

IGF-1 Modulation

Insulin-like growth factor I has been demonstrated to have an important role as a mediator in the neurogenesis of dentate gyrus granule cells in the hippocampus. It has been shown in the rat model that the treatment of IGF-1 infusions supplementing an exercise plan would increase BrdU labeling in the hippocampus [8]. BrdU (Bromodeoxyuridine), localizing to the cell nucleus, is used as a label for cells that are actively proliferating at the time of tissue collection. There was no presentation of BrdU in the experimental group that consisted of the peripheral infusion of IGF-1 with no exercise. Alzheimer's pathogenesis decreases levels of IGF-1 which contributes to the depletion of functioning neurons [9]. In this model, exercise would encourage the circulating IGF-1 to pass the blood brain barrier to rescue these compromised neurons [10]. Possibly, IGF-1 allows more efficient modulation of hippocampal neurogenesis under exercised conditions.

Physical activity has shown varying effects on peripheral IGF-1 levels. Physical activity regimens and resulting growth factor differences, have often shown males more likely to reap the benefits of increased IGF-1 following exercise [11] [12]. In elderly individuals over the age of 65 years, participation in moderate exercise resulted in increased circulating IGF-1 and cognitive function. The decline of serum IGF-1, often indicated in old age, has been shown to be accompanied by cognitive decline and neurodegeneration [13]. This evidence demands further investigation into how physical activity over a lifetime in the human model may impact circulating IGF-1 and IGF-1 receptors in the brain.

RNA Involvement in Inheritance

Recent studies have demonstrated the versatile ability of environmental factors to have a positive or negative effect on germ cells [14]. Cognitive enrichment combined with physical activity was shown in the mouse model to have an intergenerational influence in producing offspring with greater hippocampal synaptic plasticity. Intergenerational inheritance refers to the passing of traits from parent to offspring, without contribution to further generations. Environmental enrichment (EE) was used to describe the housing unit in which adult male mice were placed in prior to breeding. The EE housing unit involved objects which would stimulate physical activity through shelters, running wheels, and toys. Males were chosen as the preferred sex of focus under the study as it would eliminate maternal care variables. There was a significant increase in long-term potentiation (LTP) at the Schaffer Collateral CA1 synapse of both female and male offspring [15]. Possible confounding factors, such as paternal treatment, sex, and paternal treatment X sex interaction, were analyzed under a linear regression model and concluded the significant effect of paternal treatment. When the experiment was continued to examine the possibility of transgenerational inheritance of the acquired function, there was no enhanced LTP in the CA1 region.

The underlying mechanism of miRNAs in this inheritance pattern was examined under the same study. When identifying miRNAs to focus on under these experimental conditions, miR-132/212 was selected for having a role in cognitive function as well as intergenerational phenotype. The involvement of miR-132/212 has been found to have a correlated effect on BDNF expression [16]. This miRNA was found to have an increased presence in sperm and hippocampus of adult male mice under EE conditions. RNA of sperm from EE and control mice was injected into fertilized oocytes which generated results supporting EE mice RNA providing enriched LTP. When studying the F2 generation, there was no significant LTP carried down the lineage from F0

physical activity enrichment. The offspring did not demonstrate an increased level of miR212/132, which explains why the effect follows an intergenerational inheritance pattern. This data supports the involvement of miRNAs in exercise passing down increased cognitive function from parent to child; however, this effect would likely not be a major contributing mechanism to the evolution of the brain.

Exercise Effect on Mitochondria – Alzheimer's Model Analysis

Alzheimer's disease exists as a highly studied neurodegenerative disorder which has been shown protected by chronic exercise habits. At the cellular level, early progression of the disease can often be tracked to mitochondrial dysfunction. Advanced accumulation of amyloid precursor proteins and Aβ plaques in these organelles contributes to the pathology. Due to Aβ pathology, mitochondrial dysfunction will decrease ATP production, while increasing mutations in mitochondrial DNA [17]. Weakened mitochondria will create dangerous reactive oxygen species, without adequate rescue from the toxins. There is still much unknown about these mechanisms, but chronic exercise has shown capable of increasing the quantity of mitochondrial antioxidants. Mouse models demonstrated upregulated superoxidase dismutase and catalase in the brain after intense treadmill exercise, which rescued cells from oxidative damage [18]. Further studies regarding the effect of physical activity on mitochondrial enzyme activity could give insight to how maternal DNA affects future generations.

Sedentary Lifestyle Trends

Humans have evolved through the ability to work manual labor, while technology has suddenly depleted the need for this adaptation of energy consumption and storage. Between 2004-2006, the U.S. National Health and Nutrition Examination Survey found that 58% of non-sleeping time

was spent doing sedentary behavior for the average person [19]. Individuals reported an average of 3% of non-sleeping time dedicated to scheduled exercise. In a survey conducted by the National Center for Health Statistics Research Ethics Review Board, sedentary behaviors have greatly increased throughout modernization across the world [20]. A total of 51, 896 individuals participated after the exclusion of 669 individuals underweight, 3720 individuals with physical limitations, and 61 individuals excluded for reported sitting times of more than 16 hours per day. The demographics of participants involved child (5-11 years), adolescent (12-19 years), and adult (20+ years). Between 2007-2016, there was an increase from daily hours sitting rising from 7.0 hours to 8.2 hours. Groups of individuals that demonstrated a significant increase in this trend involved: males, non-hispanic blacks, obese, and physically inactive individuals. Majority of sedentary behaviors are found correlated to limited work and educational environments. The evolution of brain cognitive may vary by countries through their activity level and forms of exercise.

Conclusion – Proposed Loss of Cognitive Evolution

The heightened lack of physical activity will have a direct impact on brain health modulators, many of which demonstrate epigenetic inheritance. Evolution had advanced alongside physical advantage breakthroughs, many of which are not currently utilized by humans. Muscle atrophy and excess fat are visible to the eye; however, it is crucial to understand the effects of sedentary behaviors on the brain. There is an obvious demand for studies that give insight to how the lack of physical activity will continue to affect how our genome is expressed for generations to come. Current studies have shown that exercise significantly contributes to enhanced cognitive function and neurogenesis. Without exercise, the enhanced brain function was lost. Adjusted with our physical activity level, modulators of cognitive function such as brain-derived neurotrophic

factor and IGF-1 could have negative effects on our future generations of humans. Combining neurologic research with sociological studies, scientists and community agencies could endorse new policies that would ensure the continued success of human evolution. In 2018, the World Health Organization has taken steps to reduce non-communicable disease through an initiative called Sedentary Behavior Reduction under the Global Action Plan [21]. This holistic plan incorporates actions which target to create active societies, environments, people, and systems in the pursuit of reducing daily sedentary time. Strategies which are implemented by this program have been proved to be of best-practice technique. Research on brain alterations due to physical inactivity is a major contributing discipline as evidence-based science is crucial in promoting healthy social behavior and policy making. Evolution is impacted through the physical abilities of a species and adaptations to those activities. Maintaining high physical activity levels is crucial in maintaining our cognitive function.

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