# NEUROMELANIN AND NEURODEGENERATION

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Master of Arts in Human Development and Family Studies

by

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# ABSTRACT

The goal of this review was to explore the structure and function of neuromelanin and the role it plays in homeostasis and neurodegeneration by consolidating and synthesizing published scientific information in this area. This paper gives an overview of both healthy neuromelanin function and pathological neurodegenerative processes. Neuromelanin can play both a protective role and/or a damaging neurotoxic role. This review explores and highlights in more detail the roles, structure and function of neuromelanin and possible ways to alleviate the damaging effects.

## BIOGRAPHICAL SKETCH

After working and volunteering in the Mental Health field for 20 years, Andrea (Krystal) Glass completed her Bachelor of Science degree in Biological Sciences from Colorado Mesa University in 2010, minoring in Psychology with an emphasis in Chemistry. While at CMU, Krystal not only completed a thesis on infertility and new treatments, but was very involved in volunteer work as well, both on campus and in the community. She served as an officer in the student body and oversaw several community outreach programs. She completed an internship with The Charles C. Gates Center for Regenerative Medicine and Stem Cell Biology at Anschutz Medical Campus – University of Colorado, where she served as a Research Assistant for cancer and stem cell studies. Later, she attended Physician Assistant school at the University of St. Francis – Albuquerque, where she completed a second thesis entitled "A Comparative Review of the Exercise Effects of Cycling, Aquatic Therapy, and Tai Chi on Parkinson's Disease." While there, she served as the National Student Representative for her PA class. After leaving Physician Assistant school, Krystal continued her education at Cornell University to pursue her Master of Arts degree in Human Development and Family Studies. She is a member of The New York Academy of Science.

DEDICATION

This work is dedicated to my Family, to all those who have supported me in my academic endeavors, and to all those who may benefit from this research.

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## Introduction

Neuromelanin (NM) is a dark polymer pigment produced in certain catecholaminergic neurons and appears in greatest quantities in the human brain. Interest in the pigment is on the upswing in recent years due to a hypothesized link between NM and the especially vulnerable neuromelanin containing neurons to cell degeneration in Parkinson's disease (PD).[1,2] Unlike peripheral melanins, which can be transferred to other cell types and produced in melanocytes, neuromelanin granules are believed to be stored in the cell in which it was produced.[1,2,3] Neuromelanin is more heterogenous in appearance compared to peripheral melanins and is traditionally thought to result from non-enzymatic synthesis with no known pathway for catabolism. Neuromelanin is believed to function in vivo to attenuate damaging stimuli effects with the possibility of interacting with transition metals, especially iron, and to mediate intracellular oxidative mechanisms. Neuromelanin in PD brains suggest this function may be compromised, rendering pigmented neurons vulnerable to oxidative damage.[1,2,3] Neuromelanin binds iron and plays a protective and/or damaging role in brain chemistry. The role is dependent on the amount of bound iron. Dopamine is oxidized by iron and forms a quinone byproduct. These byproducts can initiate toxic pathways leading to neurodegenerative cell death.[4]

In recent years, more neuromelanin research is being conducted. Areas include Parkinson's Disease and other neurodegenerative diseases. Neuromelanin has been somewhat of a mystery to the science community. Although we have learned much about the synthesis and some of its functions, we still have much to learn about neuromelanin dysfunction and how it effects homeostasis. In this review, we will be

looking at neuromelanin mechanisms and dysfunction as well as the role it plays in neurodegenerative disorders.

**Types of Neuromelanin and Distribution**

Melanin in the brain has a similar appearance and structure to cutaneous melanins. Due to their base molecular structures, melanins are classified into four groups, which include eumelanin, pheomelanin, neuromelanin, and allomelanin. Neuromelanin is thought to be related to melanins outside of the central nervous system and to be a combination of eumelanin and pheomelanin.[1] NM is found in two types of catecholamine synthesis cells, noradrenaline and dopamine. NM in these cells is likely composed of various neurotransmitters produced in the specified neuronal cells, although this has not been proven due to the small number of cells that produce these neurotransmitters. It is thought that NM is formed by oxidative polymerization of dopamine and noradrenaline with possible involvement of cysteinyl derivatives. Neurons that produce NM are primarily found in the brainstem, the brainstem pigment in the midbrain and pons, some in the hypothalamus, medulla oblongata, the cerebellum near the fourth ventricle, and the spinal sympathetic ganglia. Except for the spinal sympathetic ganglia, these regions are involved in conscious perception, movement, emotion and memory. The group in the midbrain and hypothalamus contain dopamine, those in the pons contain noradrenaline, while those in the medulla oblongata contain noradrenaline and adrenaline.[1] Sixty-five percent of noradrenaline neurons contain NM, which suggests synthesis and use of noradrenaline. Noradrenergic NM containing neurons are important for autonomic control of cardiac and respiratory integration and regulation of hypothalamic

hormones.[1,2]

In the pons, catecholamine neurons accumulate in the locus coeruleus. Neurons in this nucleus produce noradrenaline with practically all neurons in the locus coeruleus containing NM. The locus coeruleus is involved in the activation of activity states, such as the sleep-wake cycle and the regulation of gathering and processing of sensory information during attentive state. Pigmented neurons are scattered in clusters throughout the medial midbrain and continue into the arcuate nucleus of the hypothalamus. Compared with other catecholamine cells, neurons in the substantia nigra consistently contain larger amounts of NM. Although dopamine and noradrenaline synthesis are required for NM production, the synthesis and use of the catecholamines do not ensure that NM is produced. [1,2]

**Comparison of Melanins**

Peripheral melanins are diverse in proportion and amount of eumelanin to pheomelanin. It is produced in melanocytes and either remains within the cell or is transferred to other cell types, such as keratinocytes or hair cells. NM exists as dark colored granules in cytoplasm of catecholaminergic neurons where it is synthesized. NM in non-neuronal cells is associated with neurodegeneration after toxin-induced neuron death. Although melanin and NM are found in the cytoplasm of their respective cells, the structure of NM appears very differently to that of peripheral melanins.[1,2,3] In peripheral tissues, melanins are thought to function as endogenous mediators of oxidative mechanisms. By analogy, NM may participate in a similar role within the brain. It was suggested that synthesis of NM could play a protective role within the cell by preventing the accumulation of toxic catechol derivative by

incorporating into the polymer. This could provide a chemoprotective mechanism by interacting with a variety of potentially damaging molecules, such as pesticides and toxic compounds. Epidemiologic research has linked PD development to pesticide exposure, implicating NM in the etiology of neurodegeneration in this disease. Another suggested role for NM is in the binding of metals, particularly toxic cations such as iron, zinc, copper, manganese, chromium, cobalt, mercury, lead and cadmium. [1,2,3] Neuromelanin is also known to bind neurotoxic compounds such as paraquat, chloropromasine, galoperidol, and imipramine potentially toxic for dopaminergic neurons. NM most likely plays a physiological role in intraneuronal iron homeostasis, as it strongly binds iron due to similar iron-binding sites structure being analogous to ferritin. Changes were seen in NM in the PD brain where significantly less iron is bound to NM than that seen in normal brains. This suggests that changes in iron-binding to NM result in increased levels of intraneuronal free iron and subsequent cell damage seen in PD. Many characteristics of normal biology of NM remain to be clarified, particularly the regulation of NM synthesis and turnover. For peripheral melanins, enzymatic synthesis and turnover is highly regulated. [1,2,3]

**Neuromelanin Location and Structure**

Neuromelanin is found in the pre-synaptic terminal of dopamine neurons. It is absent at birth and becomes detectable in the brain around age one.[5] It increases in synthesis during adolescence and continues to do so throughout a person's lifetime. As a result, aged brains have a darker appearance in these regions. Neuromelanin is most noticeable in the substantia nigra and locus coeruleus catecholamine neurons. NM is the major iron storage component in the substantia nigra.[5,6]

Neuromelanins are spontaneous occurring polymers structurally similar to peripheral melanins, although unlike neuromelanins, peripheral melanins are non-spontaneous and enzymatically driven. Both types have the ability to chelate and neutralize transition metals, ions, and lipids for long periods of time. Molecular structure of neuromelanin contains cysteine in its pheomelanin core, which is surrounded by a eumelanin without a cysteine component; however, it is not known exactly how the polymer is arranged.[5,6]

The fact that neuromelanins are packaged into double bound membrane structures within cells of the substantia nigra and other neural tissues suggests a beneficial function to sustaining this storage. Nicotine bound to synthetic neuromelanin created in a lab, while caffeine did not.[7] The active component of tobacco, nicotine, has surprisingly demonstrated a protective effect against Parkinson's disease in both humans and animal models. The mechanism of this effect is unknown. Protection is observed when nicotine is administered before, but not after, neural toxicity. Caffeine is also neuroprotective against Parkinson's disease. Toxic levels of 6-hydroxydopamine can damage the blood-brain barrier by increasing the permeability. The protection caffeine provides is thought to be attributed to its ability to prevent the barrier from increasing in permeability.[7] Additional studies plan to measure binding affinity of more relevant environmental elements with neuromelanin with the goal of predicting and preventing chemical exposure damage which may initiate the onset and progression of Parkinson's disease.[7] As we learn more about neuromelanin structure and function, it becomes more clear that neuromelanin is a key component in dopaminergic balance; therefore, having a direct

effect on neurodegeneration.

**Peripheral Melanin-Concentrating Hormone**

Melanin-Concentrating Hormone (MCH) is a hypothalamic neuropeptide characterized with a role in physiologic function in arousal, mood, reproduction, and energy homeostasis. It is also found to be produced in the zona incerta. Ablation of functional MCH resulted in increased energy expenditure through increased metabolic rate, increased locomotor activity, or both. MCH is expressed in the central nervous system and is found primarily in the rostral zona incerta/incerto-hypothalamic and lateral hypothalamic areas. No strong physiological effects of circulating MCH have been discovered. There appears to be some differential regulation of MCH levels between a combination of gender and adiposity. Circulating MCH was not a marker of energy homeostasis and suggests that circulating MCH may not have a signaling role. [3,8]

MCH binds to and activates MCH receptors found on the surface of plasma membranes. The $MCH_1$ receptors are additionally coupled to the Gi signaling pathway that inhibits cyclic adenosine monophosphate (cAMP) production and densely expressed in the nucleus accumbens (NAc) shell, locus coeruleus, and dorsal raphe nucleus. The receptor mRNA and protein are distributed in the hypothalamic nuclei, paraventricular nucleus, hippocampal formation, septum, and amygdala. All the aforementioned brain regions are implicated in the regulation of emotion and stress, as well as in learning and memory, suggesting that the $MCH_1$ receptor is involved in these physiological events. In contrast, $MCH_2$ receptors are expressed specifically in the brain and detected in the hippocampus and amygdala.[3,8] This could

indicate an involvement in food intake regulation and feeding behavior. Transgenic mice overexpressing MCH in the lateral hypothalamus became mildly obese and susceptible to weight gain and insulin resistance when on a high fat diet. MCH regulates secretion of adrenocorticotrophic hormone (ACTH), luteinizing releasing hormone, gonadotropins and thyroid stimulating hormone. This indicates that MCH participates in the stress response, reproductive functions and energy homeostasis by regulating neuroendocrine systems. [3,8]

**MCH in Depression and Anxiety: Behavioral and Neurochemical**

$MCH_1$ receptor is reportedly expressed strongly in the NAc shell, a region of the brain known for its role in motivation and reward. Key elements of depressive states include decreased motivation and anhedonia. Injection of a $MCH_1$ receptor antagonist in forced swim test rats resulted in antidepressant effect. This increases the possibility that the MCH system in the NAc shell is closely related to the development of depression.8 Further, $MCH_1$ receptor deficiency in mice resulted in upregulation of dopamine receptors and noradrenaline transporters in the NAc shell, indicating that $MCH_1$ receptors may mediate mesolimbic monoamine function. Regulation of mesolimbic dopamines by the MCH system is thus considered to be involved in the development of depression.[4]

Hypothalamus-pituitary adrenal (HPA) axis dysfunction has been seen in patients with major depressive disorder, anorexia nervosa, and post-traumatic stress disorder. MCH neurons project into the PVN of the hypothalamus, strongly implicating a HPA axis-related stress response. $MCH_1$ receptor mRNA and protein are abundant in this area. Serotonergic transmission in the prefrontal cortex is

reported to be related to the modulation of anxiety and fear.[8] Serotonin efflux increases in the prefrontal cortex in animals when exposed to conditioned fear stress. However, $MCH_1$ receptor knockout mice have reduced basal extracellular serotonin levels in the prefrontal cortex. Wild type mice used in the forced swim test had a marked increase in serotonin release in the prefrontal cortex, while this increase was blunted in MCH1 receptor null mice. This suggests that disinhibition of serotonergic transmission by the MCH/$MCH_1$ receptor signaling may lead to anxiety like behaviors.[8] Additionally, an $MCH_1$ receptor antagonist, chlordiazepoxide, has been reported to block the stress induced release of acetylcholine in the prefrontal cortex. As the prefrontal cortex is believed to be the area that regulates neuroendocrine responses to stress, this effect could therefore be involved in the anxiogenic effect mediated through the $MCH_1$ receptors. Knockout mice lacking MCH display antidepressant-like behavior (reduced immobility) in the forced swim test, also indicating that lack of MCH may cause depressive like behavior. Efforts to delineate molecular mechanisms underlying antidepressant and anxiolytic effects of $MCH_1$ receptor antagonists have just begun, and these studies will provide a clearer picture to better understand the role of the MCH system and the molecular mechanisms of depression and anxiety.[8]

**Neurodegeneration and Neuromelanin**

Neuromelanin binds iron and plays a protective and/or damaging role dependent upon the amount of bound iron present. Dopamine oxidized by iron produces DA-o-quinone, which initiates neurodegenerative toxic pathways formed with amino acid residues. Amino acid residues are made up of mainly cysteine

residues or different proteins. On the other hand, cytosolic DA-o-quinone could react with cysteine to form cysteinyl-DA compounds, the precursor to neuromelanin synthesis.[4] It is of interest to note that neuromelanin itself has a eumelanin component comprised of a cysteine core. Neuromelanin accumulates in dopaminergic neurons of the substantia nigra throughout the aging process. It appears that iron, dopamine, and neuromelanin interact through multiple pathways. A common neurodegenerative disease marker is impairment of iron homeostasis, which is effected by several factors. These would include aging, mitochondrial dysfunction, oxidative stress, protein aggregation, etc.[4]

Increased accumulation of iron in specified brain regions, including the substantia nigra, is normal in aging; however, it is enhanced in many neurodegenerative disorders. The age-related iron accumulation may be contributing to the neurodegenerative processes. This is often associated with oxidative stress and cellular damage. In neurodegenerative diseases, such as Parkinson's, these same brain regions are less pigmented with much lower levels of neuromelanin. The loss of substantia nigra neuromelanin is seen in all Parkinson's patients. The neurodegenerative process in Parkinson's is initiated years before the appearance of motor symptoms become present. One symptom of Parkinson's includes depression.[4] The current scientific general consensus is that of multiple factor involvement resulting in the loss of neuromelanin dopaminergic neurons during Parkinson's. Some of which include neuroinflammation, oxidative stress, protein degradation dysfunction, endoplasmic reticulum stress, and mitochondrial dysfunction, to name a few. It is important to remember that the formation of a-quinones by dopaminergic

oxidation occurs inside the neurons that are lost during the disease. These a-quinones can participate in both neurotoxic and neuroprotective reactions. It is most likely that cell death is induced by the a-quinones during dopaminergic oxidation and is a main event that explains why neurodegeneration is a very slow process in Parkinson's.[4]

The synthesis of neuromelanin itself is a protective process which is sustained by excess dopamine in the cytosol and not accumulated by synaptic vesicles. Dopamine in the cytosol can be oxidized to create DA-o-quinone by iron mediated catalysts and then follow one of two different pathways: eumelanin or pheomelanin. Without neuromelanin synthesis, dopamine accumulation in the cytosol would induce the neurotoxic effects previously described.[4]

Neuromelanin possesses two types of iron binding sites. In neurodegenerative conditions of iron overload, such as with Parkinson's, the high affinity sites of neuromelanin would be saturated. Iron would only be able to bind to low affinity sites. If this were to occur, iron is bound into a low stability complex and would be easily released, becoming toxic by participating in redox processes and oxidizing ascorbate, a main antioxidant system in the brain. Neuromelanin iron overload can also catalyze dopamine oxidation and formation of further DA-o-quinone, which induces oxidative modification of proteins, unleashing a neurodegenerative cascade.[4]

When neuromelanin iron overload is present, the rate of neuromelanin degradation by hydrogen peroxide increases as well. This is likely to occur during microglia, when neuromelanin released by dying neurons is phagocytosed and broken down, so reactive iron and other toxins previously accumulated by neuromelanin are suddenly released and can cause cell death. Neuromelanin likely contributes to

neurodegenerative progress by contributing to a neuroinflammatory process that involves activation of microglia. Increased levels of redox-active iron have been reported in neuromelanin of the substantia nigra from Parkinson's patients.[4] A common feature of Parkinson's is neuromelanin release from dying neurons in the extracellular space of the substantia nigra, along with increased microglial activity. Neuromelanin granules released into the extracellular space of the substantia nigra would most likely remain for long periods of time due to the level of granule insolubility. The extra-neuronal neuromelanin granules would serve as a chronic source of neuronal inflammation. A protective role of neuromelanin has been hypothesized when iron is bound under these physiological conditions, but a toxic effect of neuromelanin iron complexes occurs when iron overload is present in the brain.[4]

In the last thirty years there has been increasing evidence that dysregulation of cytosolic dopamine and reactive metals, particularly iron, plays an important role in the pathogenesis of Parkinson's Disease. Neuromelanin blocks reactive iron and forms stable complexes preventing iron toxicity. When conditions are disturbed, iron and neuromelanin initiates a series of toxic neuroinflammatory steps that creates a 'vicious cycle' sustaining the progression of the disease.[4]

It was recently suggested that neuromelanin plays the role of inducing alpha-synuclein (a-syn) expression and accumulation, which is another way neuromelanin is suggested to regulate neurodegeneration in Parkinson's. Pathological neuron death induced by a-syn could occur through various mechanisms, including oxidative stress and preventing protein degradation. What causes a-syn to convert from a non-toxic

protein into a toxic one is unclear. Some causes for toxic a-syn accumulation may include missense mutations in the a-syn gene, higher concentration and posttranslational modification of a-syn. Neuromelanin induces a-syn expression in the dopaminergic neurons of the substantia nigra in aged and Parkinson's brain.[9] Neuromelanin binds directly with a-syn in lateral areas of the substantia nigra neurons in Parkinson's patients. Since a-syn is expressed in melanoma and nevus, but not in non-melanocytic cutaneous carcinoma and healthy skin, it would suggest that melanin may induce the expression of a-syn. Also, a-syn mRNA expression was significantly higher in individual neuromelanin containing dopaminergic neurons in Parkinson's patients than those of healthy controls.9 This finding further supports the claim of neuromelanin induced expression of a-syn. Neuromelanin also increases the accumulation of a-syn in dopaminergic neurons. The increased levels of a-syn increased the levels of neuromelanin in dopaminergic neurons, which suggests a-syn may promote the biosynthesis of neuromelanin in dopaminergic neurons. A-syn plays a key role in the pathogenesis of Parkinson's Disease.[9] Although Parkinson's is not commonly considered genetic, a missense mutation in the a-syn gene could cause early onset familial Parkinson's. Abnormal a-syn toxicity forms to dopaminergic neurons and normal function loss of the protein are important causes of the pathogenesis in sporadic Parkinson's. The study suggests that interaction between neuromelanin and a-syn is the mechanism for modulation of neuronal susceptibility. Increased a-syn in individual melanized neurons and its accumulation redistributed to neuromelanin in the substantia nigra early in Parkinson's disease, but did not do so in healthy controls. Alternatively, a-syn induces the biosynthesis of neuromelanin by

increasing the levels of cytosolic dopamine. The conclusion of this particular study stated that neuromelanin is responsible for Parkinson's disease and age-related increase of accumulated a-syn.[9]

It is of interest to note that 66% of Parkinson's patients report having motor symptom onset on their right side first. Almost all patients with Parkinson's show both clinical and imaging asymmetry. Handedness may correlate with motor symptom asymmetry.[10] The left hemisphere could very well have greater potential for neurodegeneration as well. However, the presence of imaging asymmetry in healthy controls suggests that neuromelanin lateralization may not be specific to Parkinson's. It is unknown whether this is an innate occurrence, or if it is a consequence of the aging process.[10]

One of the main differences between catecholamine neurons in some mammalian species is the presence or absence of neuromelanin in the cytoplasm. This would be important to know for some comparative neurobiological studies, especially with the use of lab animals. It is not known why neuromelanin is absent in some mammalian species. Catecholamine neurons are separated into three different categories: dopaminergic, noradrenergic, and adrenergic.[2]

Neuromelanin is a result of catecholaminergic oxidation, including norepinephrine (noradrenaline) in the Locus Coeruleus. Neuromelanins also remove excess catecholamines from within a cell. Imaging studies optimized to detect neuromelanin are showing greater signal in the Locus Coeruleus in older adults is associated with higher verbal knowledge. Lower signal is associated with Alzheimer's disease and minor cognitive impairment.[11]

Animal studies are showing norepinephrine helps protect neurons from damage that can cause neurodegeneration with age, such as excitotoxicity and neuronal inflammation. Introducing norepinephrine to the hippocampus of older rats reversed age-related long-term possible deficits. The increase in norepinephrine improved cognition in the aging rats and in transgenic mice models of Alzheimer's. Norepinephrine plays a key role in age-related cognitive ability and appears to effect attention and memory function as well.[11] The Locus Coeruleus is the brains key source of norepinephrine.[12]

Brain and cognitive reserve reference an individual's ability to manage cognitive decline or disease later in life. The idea of cognitive reserve has created much interest in the field of aging, especially with Alzheimer's. Aspects believed to encourage reserve not only predict lower risk of dementia but also mask the severity of underlying neurodegenerative diseases.[12] Cognitive reserve capacity is thought to be dependent upon physical neural resources and higher cognitive reserve scores. Scores are based on an individual's level of education, occupation, and IQ. Neuromelanin signal intensity was associated with cognitive reserve, especially in those with higher verbal intelligence. Elderly individuals with low levels of cognitive reserve showed greater association with locus coeruleus neuromelanin intensity levels and attentional shifting.[12] This indicates that the integrity of the noradrenergic system supports cognitive flexibility. Neuromelanin signal in the Locus Coeruleus was much higher in older adults than younger adults and lower in women than in men. These findings lend support to the idea that by enhancing the locus coeruleus norepinephrine system function and an individual's enriching life experiences might help to protect

cognitive health later in life.[12]

**Neuromelanin and Mood Disorders**

Iron is a co-factor of tyrosine hydroxylase, which converts tyrosine into dopamine and then later into norepinephrine.[13] Dopamine regulates cellular iron homeostasis by increasing iron into macrophages and later promotes intracellular oxidative stress. Other disorders this research may be of interest to not only includes neurodegenerative disorders but dopamine dysfunction disorders as well, such as restless legs syndrome. Specifically, one may note the pathogenesis of these disorders or the efficacy of dopamine agonist therapy to overcome neuronal iron deficiency.[13]

Dopamine dysfunction plays an important role in the pathogenesis of schizophrenia.[14,15] Further research is being conducted specifically in the area of dopaminergic pathways and the substantia nigra. Antipsychotic drugs currently used to treat schizophrenia function through blocking dopamine receptors. Patients with Parkinson's being treated with dopamine-enhancing compounds, such as levodopa, may experience psychotic episodes which mimic the symptoms of schizophrenia. The substantia nigra is a major origin of dopaminergic pathways. Image studies have shown more dopamine is produced in the substantia nigra of schizophrenic patients. Increased levels of tyrosine hydroxylase were also detected, indicating increased levels of dopamine synthesis. The Substantia nigra in schizophrenia is hyperactive, which is linked to the prefrontal cortex hypofunction of the disease.[14]

Increased levels of neuromelanin are being seen through MRI in the substantia nigra of schizophrenic patients. This finding indicates the existence of increased levels of dopamine production in the substantia nigra. Neuromelanin is now being

used as a signal or biomarker for researching dopaminergic and noradrenergic pathways in schizophrenia. Neuromelanin MRI imaging has the potential to be useful for schizophrenic diagnosis.[15]

The substantia nigra area of the brain plays an important role in reward and movement. One study researching the link between neuromelanin levels in the substantia nigra and psychosis severity found higher levels of neuromelanin to be associated with increased severity of psychosis. Schizophrenic psychosis is associated with dopamine dysfunction, characterized by increased levels of dopamine and dopamine synthesis.[16] The use of neuromelanin MRI imaging allows more research to be conducted in disorders beyond those that are neurodegenerative. The ability to measure neuromelanin concentrations and capture dopamine function in the substantia nigra will contribute to research in other areas. Some such areas include looking to see if abnormalities in neuromelanin can be detected and assist in predicting if an individual is more likely to develop a psychotic disorder among those that already show early symptoms of psychosis. Further exploration is also being conducted in whether neuromelanin MRI could be used to determine if an individual would best benefit from dopaminergic treatment.[16]

Tyrosine hydroxylase is the enzyme that limits the rate of norepinephrine production. Norepinephrine promotes hypothalamic-pituitary-adrenal activity. One study examined the levels of tyrosine hydroxylase in the locus coeruleus of mood disordered patients. Higher locus coeruleus neuromelanin activity was seen in patients with suicidal depression, especially those with violent suicidal behaviors.[17] Tyrosine hydroxylase was present in the cytoplasm and neuron fibers containing neuromelanin

in both controls and mood-disordered patients. The locus coeruleus was found to be greatly activated in major depressive disorder patients but not in Bipolar disorder patients. There was no increase of tyrosine hydroxylase in Bipolar disorder patients. This would indicate a different type of locus coeruleus activity between these two subtypes of mood disorders. Different stress-related molecular changes in the locus coeruleus contribute to the different clinical symptoms of major depressive disorder and bipolar disorder. Dysfunction of the norepinephrine system supports the pathogenesis of mania-associated disorders.[17]

**Protective Role of Exercise on Stress System Dysregulation**

Stress is defined as a reaction to various real or perceived stimuli that threatens to disrupt the homeostasis of the organism. When under threat, the human body elicits a set of neuroendocrine responses that enables them to cope with the demands of homeostatic challenge. This adaptation is mediated by activation of the stress system, which includes the HPA axis and the sympathetic nervous system (SNS). This increases secretion of glucocorticoids (GC) and catecholamines respectively. Neuroendocrine response to stress is the mobilization of lipids from adipose tissue and glucose from the hepatic glycogen stores.[18] Together with the development of an insulin resistant state in skeletal muscles ensures adequate energy supply to the brain, facilitating cognitive and physical adaptation for the "fight or flight" response. This adaptation is a controlled deviation from homeostasis and has been termed allostasis, which means "maintaining stability through change." The immediate effects of allostasis are mostly protective for the organism, but in the long-term, overactivation is associated with the development of chronic disease or risk of mental illness.

Allostatic load is mainly determined by two factors, one being individual perception of a stressful situation or "mental fitness," and two is the general physical condition of the individual or "physical fitness." Regular physical activity may have a protective effect on alleviating allostatic load and its health consequences.[18,19]

Today's stressors are more likely to be psychological in nature, such as emotional, social, and professional, rather than physical. The stress response to a psychological stressor is similar to the physical challenge response and includes activation of the HPA axis and the SNS. However, psychological stress is different in that it does not require an increased metabolic demand and there is no clear beginning and end. Chronic elevation of GC levels from persistent stress may have a negative effect on hippocampal structure and function, producing deficits in both memory and cognition. Chronic stress produces deleterious effects on the brain, such as high levels of GC decrease the number of apical dendrite branch points and produce cell loss in the hippocampus. [18,19] Chronic stress decreases levels of brain derived neurotropic factor (BDNF) in the hippocampus of rodents. BDNF regulates the survival and differentiation of neurons in the CNS and regulates synaptic plasticity. Individuals suffering from posttraumatic stress disorder have shown to have hippocampal atrophy. The hippocampus is involved in memory function and is important for "context" memory, the times and places of events that are emotionally laden. The hippocampus also regulates the stress response and inhibits the response of the HPA axis to stress. These neuronal structures are also involved in the control of the emotions and the stress response. It is unknown if stress causes permanent alteration of these neural structures within the limbic system or if it leads to persistent stress reaction and

allostatic responses.[18,19]

Exercise is "the training of the body to improve its function and enhance its fitness." The benefits are derived from an indirect action of exercise through stress reduction as well as effect on various metabolic functions of the body. Aside from the protective effects of exercise on physical and metabolic aspects related to stress, psychological and cognitive benefits have also been reported. These benefits include improvements in depression and anxiety scores and general improvement in mood and cognitive function. Results from detailed meta-analyses show that aerobic exercise is associated with reduction in anxiety scores, although the results were not uniform across the various outcome measures.[18,19] Biological components may explain the beneficial effect on depression symptoms derived from research showing that exercise promotes the secretion of neurotransmitters, like serotonin and dopamine. Regular exercise alleviates the depressive state and reduces urine cortisol amounts and epinephrine excretion. These findings suggest that regular exercise has beneficial antidepressant and anxiolytic effects associated with decrease in the neuroendocrine response to stress. In a study of over 32,000 people, it was discovered that less physically active individuals were twice as likely to report high stress levels while physically active individuals were less reactive to psychological stressors. Although there was a dose-response effect between physical activity and psychological well-being, the most prominent effect within the level of stress and dissatisfaction was seen between the group with low and moderate physical activity.[18,19]

With there being inconsistency between studies, the overall evidence strongly suggests that sensitivity to stress is reduced after exercise training.[18,19,20] Factors that

modulate the degree of stress response include the duration of the stress exposure, perceived controllability of the stressor, and physiological state of the organism. Exercise may provide a way to control these factors and lead to adaptive effects on the HPA axis and SNS by lowering cortisol and catecholamine levels as well as reduced cardiovascular and SNS response to stressful stimuli. Exercise also increases endogenous opioid activity in the CNS and peripheral nervous system, contributing to a stress buffering effect of euphoria, analgesia and mood improvement. Physical activity produces CNS adaptations that prevent or ameliorate deleterious effects of chronic stress on mental and physical health. [18,19,20]

**Exercise Down-regulation of MCH**

As previously discussed, chronic stress is a risk factor for anxiety disorders. Chronic and repeated stress in animal models induces anxiety-like behaviors in various behavioral tests, lasting for up to three months after the stressor was removed. These studies in animal models revealed that neurotransmitters, including GABA, monoamines, cannabinoids, neuropeptides, neurotrophins, and cytokines regulate anxiety states.[20] Repeated stress up-regulated MCH in the hippocampus, amygdala, and lateral hypothalamus, the areas important for emotional behaviors.[20] Exercise downregulates the stress induced increase of MCH and affords long lasting anxiolytic effects. It was speculated that corticosterone increases from stress stimuli induced the up regulation of MCH in the hippocampus. When in vitro experiment of the HT22 hippocampal cell line was treated with corticosterone, MCH expression increased significantly. The corticosterone treated mice were then introduced to scheduled exercise to determine if the exercise would reverse the MCH upregulation in the

brain.[20] MCH up-regulated expression in the hippocampus, basolateral amygdala and lateral hypothalamus were reversed in the animals exposed to the scheduled exercise treatment. These changes in MCH expression are consistent with the idea that exercise, as a treatment for stress, has an opposing effect on anxiety-like behaviors. Corticosterone treatment induced MCH mRNA expression supports the possibility that up-regulation of MCH expression was initiated by corticosterone. Finding that stress increased MCH expression, while exercise reversed the effect, supports the hypothesis that repeated stress produces anxiety-like behaviors by upregulating MCH in the brain while exercise counteracts stress-induced MCH expression and stress induced anxiety-like behaviors. [20]

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