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Detrimental effects of physical inactivity on neurogenesis

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Abstract:

Patients diagnosed with neurological disorders exhibit a variety of physical and psychiatric symptoms, including muscle atrophy, general immobility, and depression. Patients who participate in physical rehabilitation at times show unexpected clinical improvement, which includes diminished depression and other stress-related behaviors. Regenerative medicine has advanced two major stem cell-based therapies for central nervous system (CNS) disorders, transplantation of exogenous stem cells, and enhancing the endogenous neurogenesis. The latter therapy utilizes a natural method of re-innervating the injured brain, which may mend neurological impairments. In this study, we examine how inactivity-induced atrophy, using the hindlimb suspension model, alters neurogenesis in rats. The hypothesis is that inactivity inhibits neurogenesis by decreasing circulation growth or trophic factors, such as vascular endothelial growth or neurotrophic factors. The restriction modifies neurogenesis and stem cell differentiation in the CNS, the stem cell microenvironment is examined by the trophic and growth factors, including stress-related proteins. Despite growing evidence revealing the benefits of "increased" exercise on neurogenesis, the opposing theory involving "physical inactivity," which simulates pathological states, continues to be neglected. This novel theory will allow us to explore the effects on neurogenesis by an intransigent stem cell microenvironment likely generated by inactivity. 5-bromo-2-deoxyuridine labeling of proliferative cells, biochemical assays of serum, cerebrospinal fluid, and brain levels of trophic factors, growth factors, and stress-related proteins are suggested identifiers of neurogenesis, while evaluation of spontaneous movements will give insight into the psychomotor effects of inactivity. Investigations devised to show how *in vivo* stimulation, or lack thereof, affects the stem cell microenvironment are necessary to establish treatment methods to boost neurogenesis in bedridden patients.

Key words:

Immobilization, neurogenesis, neurological disorders, physical exercise, stem cells

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A Call to Examine Stem Cell Effects with Physical Inactivity

Our encompassing hypothesis is absence of exercise impacts the neurogenic niche in the brain, thereby modifying the stem cell microenvironment. Until a short time ago, the nonregenerative ability of the adult injured brain was accepted as a scientific creed. Yet, growing evidence over the last decade reveals that neurons and astrocytes can be produced from isolated cells of the adult mammalian central nervous system (CNS).[1] Shortly after, several laboratory studies investigated stem cell therapy for treating numerous diseases in the CNS, including stroke, traumatic brain injury, and neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease. Stem cell therapy, nonetheless, continues to be considered an experimental treatment. Countless patients continue to deteriorate because of these diseases, and the number of bedridden cases is rising. Bedridden patients' muscles begin to atrophy, their everyday activities are reduced, and some patients begin to present a depressive mood. In addition, patients who are able to attend rehabilitation in the clinic occasionally show remarkable clinical improvements, along with reduced depression and a diminishment of other stress-related behaviors. Unfortunately, there is a scarce amount of information available regarding the effects of disuse atrophy on the innate functions of the brain including neurogenesis.

Regenerative medicine is a new scientific field that has advanced stem cell therapy for the purpose of treating brain disorders, with a focus on either transplanting exogenous stem cells or amplifying endogenous stem cells through neurogenesis.[2-13] Our envisioned research project is directed toward the latter method, which utilizes an inherent technique of mending

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the injured brain and repairing the neurological deficiencies through physical rehabilitation. Within this study, we are interested in elucidating if inadequate exercise-induced disuse atrophy, via the hindlimb suspension (HS) model, will alter neurogenesis in adult rats. The relationship between inadequate exercise and neurogenesis stands to be identified; compared to the inverse model of elevated exercise levels.[14] Specifically, exercise has been demonstrated to activate neurogenesis.[14-16] Furthermore, various conditions, such as cerebral ischemia, have been shown to upregulate neurogenesis.[17] The theory of augmented neurogenesis achieved through exercise leads to the overarching concept of our thesis that inadequate exercise inhibits neurogenesis possibly by decreasing circulating factors, such as vascular endothelial growth factor (VEGF) or brain-derived neurotrophic factor (BDNF). Laboratory studies are justified to unveil the underlying biological mechanism of neurogenesis. The comprehensive observations associated with these studies will allow the development of treatments designed to further neurogenesis in patients that demonstrate a lack of mobility.

Adaptation of Hindlimb Suspension to Assess Stem Cell Therapy

The hindlimb suspension model

The HS model was originally suggested for analyzing spaceflight-associated phenomena,[18] due to the initial evidence which showed an inability for bone formation during spaceflight.[19] Following the initial use, the model has been subjected to different modifications,[20] along with bone formation studies,[21] analysis of muscle,[22] and vascular system of the hindlimbs[20]. Thus far, a majority of studies have been directed toward the peripheral response caused by the HS model.[23-25] There has only been a handful of studies which use the HS model to measure changes in the CNS, with a majority targeting depression.[26,27] In 2005, Dupont *et al*. determined that neuronal growth factor (NGF) and BDNF mRNA and also NGF protein are upregulated in the somatosensory cortex of animals placed in the HS model.[28] These documented changes in neurotrophic factor levels reinforce the idea that "exercise,"[14,15] or lack of, controls neurotrophic factor expression. Even though there has been no analysis of HS model, figure 1, in CNS disorders, there has been evidence of forelimb disuse and overuse models in stroke and Parkinson's disease.[29-32] Forced disuse (using one-sleeved casts) in stroke rats, but not overuse, of the afflicted forelimb during the early phases of recovery restricts the functional result of the healing.[33] Within rats that had somatosensory cortical lesions, involuntary overuse of the injured forelimb within the initial phases of repair was related to a decreased functional outcome from rehabilitation.[34] Led by Schallert and Jones, a group reported the aforementioned contrasting results and continued to explain that there are fundamental differences in the effects of forelimb disuse and overuse that may be resulting from the site-specific anatomical changes (e.g., subcortical in stroke versus cortical in the somatosensory cortical lesion model) that are produced by different injuries.[35] Subsequently, subcortical injury responded to the involuntary use of the affected forelimb, unlike the cortical injury. Involuntary use of the affected forelimb directly following unilateral 6-hydroxydopamine (6-OHDA) lesions in rats diminished behavioral deficits and the involuntary forelimb exercises preceding the 6-OHDA lesions boosted neurotrophic factor glial cell-derived neurotrophic factor levels from glial cells guarding against cognitive deficiencies.[36,37] The recognized reduction of behavioral deficits within stroke and Parkinson's disease animal models coincides with decreased neuroanatomical damage and fewer neurochemical deficiencies.[29-32,38-40] Through these studies, the practice of enforcing or denying a regimen of physical therapy reveals the effects on the CNS involving these diseased conditions. With these results, we can validate the effects that controlling physical therapy has on the CNS via the HS model.

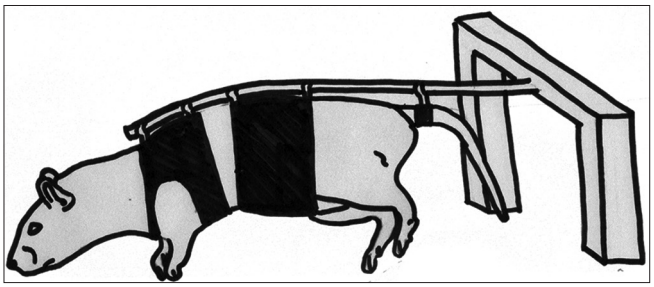

Figure 1: *The hindlimb suspension model is aimed to withhold the movement of posterior extremities for periods of time by suspending the rear limbs. This allows for observations to be drawn between experimental groups with various allotted times in the hindlimb suspension model*

Function of neurogenesis

Stroke can be the result of either a blockage located in an artery or a hemorrhage of a blood vessel within the cerebrovascular system.[41] Discreet brain areas shown to exhibit cell proliferation during adulthood are labeled as neurogenic niches.[41] Neurogenesis is defined as the proliferation and differentiation of neural stem cells into neurons.[41] The two main neurogenic niches are the sub-granular zone (SGZ) and sub-ventricular zone (SVZ) of the dentate gyrus (DG), where neural stem cells accumulate and develop into mature neurons.[41] However, neurogenesis is not limited to these locations, and recent research has shown neurogenesis in the vicinity of the peri-impact area, which can become an active sight of neural repair following a stroke.[41] The proposed mechanism of the HS model is to stimulate these neurogenesis niches to facilitate neural repair.[42] Following stroke, there is documented acute endogenous neurogenesis, allowing cells to proliferate in these neurogenic niches, thereafter facilitating the migration of neuroblasts toward the peri-impact area.[41] Because endogenous neurogenesis by itself is not sufficient to sustain neural repair, the HS model aims to increase the proliferation of the neural stem cells within neurogenesis niches, as well as enhance the migration of neuroblasts to the peri-impact area, altogether improving neural repair outcomes.

Neurogenesis, exercise, and growth factors

During innovative studies concerning exercise-induced neurogenesis, conducted by van Praag *et al*.,[14,43] rats were allowed a running wheel for 3 h during their active period and it resulted in substantial increases of 5-bromo-2-deoxyuridine (BrdU) labeled newly developed cells within the SGZ.[44] This optional use of exercise correlated with a diminished threshold for long-term potential (LTP) in the DG, concurrently increasing LTP, which conveys the theory of exercising improving

memory.[43,45] Increased levels of VEGF[15,17] and BDNF[45-48] following exercise-induced neurogenesis give the appearance of these growth factors playing a role in exercise and neurogenesis. Within the SVZ and DG, the increase of neurotrophic factors reveals an association with the microenvironments of these familiar neurogenic sites. Due to the ability of diffusion for these growth factors, the effect of exercise-induced neurogenesis is not limited to specific neurogenic sites. In a recent study,[49] the posterior hypothalamic area (PHA) exhibited modified activity pertaining to *in vitro* and *in vivo* spontaneous firing rate of PHA was noticeably diminished from the rats that exercised in comparison to the nonexercising rats. To this end, exercise-induced neurogenesis has been recognized in neurogenic sites, and potentially may stimulate neurogenesis-like neuronal activity in other non-neurogenic brain areas. In light of these results, rats with the ability to exercise have been shown to have neurogenesis in neurogenic site, but these results may also prompt similar neurogenesis effects in non-neurogenic areas of the brain. The relationship between different levels of exercise and neurotrophic factor levels indicate that introducing a regimen of exercise should result in increased levels of neurotrophic factors as well as neurogenesis. Accompanying aging is a declining capability to exercise along with the advancement of several debilitating CNS diseases, which can provide a basis to investigate the effects of lack of exercise on neurotrophic factors along with neurogenesis. Although a lack of physical activity has been linked to a wide number of health issues (e.g., osteoporosis, obesity, and cardiovascular diseases),[50-53] there has not been an in-depth look at the effect of reduced exercise on neurotrophic growth factors and neurogenesis. In order to further the research involving the effects of lack of exercise and develop physical therapies to improve the behavioral deficits caused by an inability to exercise, making it vital to discern the essential growth factors that are available at sites of neurogenesis and how they are affected by the lack of exercise. Physical activity inhibited by a debilitated state triggers motor and cognitive functions, which intensifies behavioral deficiencies within the debilitated state.[54,55] By creating a scope of research that evaluates only specific conditions surrounding physical inactivity, it would allow preliminary rehabilitation techniques to be developed to not only attempt to prevent early brain degeneration, but also promote healing from brain injuries.

Neurogenesis and stress proteins

Neurotrophic growth factors along with stress proteins are the fundamental indicators of the effects of physical inactivity, which allows the perception of both ends of the spectrum. In theory, a decline of neurotrophic growth factors and elevated stress proteins should be the result of physical inactivity and lead to decreased neurogenesis. Through trials of the HS model, it revealed that chronic stress positively correlates with physical inactivity.[27] Increased signs of depression along with chronic stress are related to increases in glucocorticoids and reductions of serotonin.[56-58] Within the aging subjects, they displayed increased glucocorticoids and reduced insulin-like growth factor-1 (IGF-1).[59] Throughout the chronic stress and aging samples, both presented with reduced neurogenesis, drawing attention to the function of IGF-1 in neurogenesis.[58] Using a similar reference, the samples examining depression and chronic stress revealed that BDNF and serotonin play a part in neuronal plasticity.[60-63] Through these prior evaluations, the chronic stress often follows the use of the HS model should reveal key data that shed light on the function of stress on neurogenesis.

Neurological effects of deficient exercise

When patients with limited mobility are restricted in their regiment of physical activity, it hinders the ability of a full clinical recovery. Thorough research has presented evidence that consistent exercise encourages endogenous neurogenesis and may also have a preventive measure against CNS disorders. In the past, we have examined the effects of limited physical activity relating to neurogenesis using the HS model for a 2-week stint. The HS model procedure involves lifting the rat by the tail, therefore raising their hindlimbs and transfers the weight to the forelimbs. The exercise and recovery time for the rats that were returned to a normal caging environment following HS were assessed as well. Rats received an injection of BrdU (50 mg/kg, i.p.), which is a chemical used as a marker for proliferative cells, every 8 h for the remaining 4 days of each treatment group. Immunohistochemistry results revealed that HS dramatically reduced the levels of BrdU/doublecortin (Dcx) double-positive cells within the SVZ as well as the DG zone of the brain. Although atrophy of the soleus muscle was reduced through exercise and a recovery period, the reduced levels of BrdU/Dcx-positive cells did not restore to pre-HS levels. Another similar group of rats was given an identical HS treatment along with the addition of an enzyme-linked immunosorbent assay (ELISA) of neurotrophic factors that were administered on the brain tissue, which was collected following the completion of HS treatment. Furthermore, plasma from all animals treated was administered ELISA assays of neurotrophic factors. The results imply that the levels of natural BDNFs within the hippocampus as well as VEGF plasma levels were reduced by the treatment of the HS model. Through this experiment, it has been revealed that a reduced exercise regimen following brain injuries reduces neurogenesis due to lower levels of neurotrophic factors within the brain. By combining the HS model with the CNS disease models, the effects of various levels of physical activity on neurotrophic factors and neurogenesis can be evaluated further.

Rehabilitation and neurogenesis

Previous research studies have identified that reduced physical activity can lead to a variety of health complications (e.g., osteoporosis, obesity, and cardiovascular diseases).[50-52] The functions of neurogenesis and neurotrophic factors have yet to be assessed under these circumstances of reduced exercise. When limitations reduce the amount of physical activity, a recovering patient can participate in; it creates atypical cognitive and motor functions, which can affect the patient once they resume a normal healthy state of life. When a recuperating patient lacks physical activity, it exacerbates the behavioral deficits.[54,55] Within clinics, the positive effects of increased exercise are noted, although some therapy regimens are not proven and have shown few results. Likewise, an 18-day regimen of forced treadmill physical therapy showed no recovery progress involving memory and motor functions concerned with the upregulation of BDNF mRNA in CA1 and CA3, but not DG.[64] Similarly, the process of immobilizing a nonimpaired limb using a cast to further the use of the impaired limb following an injury

of the sensorimotor cortex, the restraint, in fact, diminishes co-ordinated movement of both limbs.[35] However, there is substantial evidence that physical activity reduces the neuronal damage and motor function in rodent neurological disorder models.[65] The multitude of rehabilitation strategies that have various durations, regularity, and other intricacies along with the level of severity of the patient's condition can have an effect on the data. These variables can lead to difficulty replicating for future research. The standard recovery phase of 2–4 weeks and post-HS physical therapy for 2 weeks reduced the inflammation of the soleus muscle, so it neared to a normal level. On the contrary, when the HS-impaired neurogenesis was removed in the SVZ and DG, it was not improved by recovery and exercise, revealing the need for more development of the rehabilitation through physical therapy. Our study sheds light on the possible roles of neurotrophic factors that could help determine therapeutic candidates for reversing the physical inactivity due to behavioral deficits. Specifically, neurotrophic factors, BDNF and VEGF, show involvement with neurogenesis.

Conclusions

We have analyzed a novel paradigm – the HS model, to investigate the effects of physical inactivity on neurogenesis in the adult brain. The basis for concentrating on neurotrophic factors along with other stress proteins to monitor the effects of physical inactivity on neurogenesis is derived from the widely accepted theory of "increased exercise" or "enriched environment," also from successful trials of the HS model in peripheral injury (bone and muscle). Besides this being the 1st time the HS model is incorporated into the CNS model (i.e., neurogenesis), the novel scientific development in this designed study is our aspiration to offer a more accurate approximation of aging and diseased brain states, where physical inactivity is a major characteristic. As a result, the physical inactivity paradigm will present new information regarding neurogenesis that would have been otherwise overlooked during the increased exercise and enriched environment models. Preclinical studies are a necessity to evaluate the possible modifications in neurogenesis within the models of immobilized rats. Furthermore, biomarkers for stem cell alterations have to be achieved in which they are essential to include growth factors and stress-related proteins, anticipating the alterations in neurogenesis caused by physical inactivity.

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Conflicts of interest

There are no conflicts of interest.

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