DEVELOPING AN ASTROCYTE SIGNALING MODEL TO INFORM AND IMPROVE STROKE TREATMENT

ETHICAL CONCERNS OF BIOMEDICAL RESEARCH FUNDING

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By
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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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In 2017, stroke was the fifth leading cause of death in the United States (Murphy et al, 2018). There are three main types of strokes: an ischemic stroke, a hemorrhagic stroke, and a transient ischemic attack (NIH, 2019). An ischemic stroke, which comprises 87 percent of stroke incidences, occurs when there is a blockage in a blood vessel that is supplying the brain, resulting in cell death as oxygen and nutrients cannot reach brain cells (NIH, 2019). Every year, more than 795,000 Americans have a stroke, and in 2014-2015, stroke-related costs in the US were more than 46 billion dollars (CDC, 2020). The National Institute of Health (NIH) funds a great portion of biomedical research, including strokes. This technical project focuses on a possible target for stroke therapy. While this investigation is underway, the ethical concerns of research funding have become an issue for labs across the country, and must be considered. For decades the US has led the world in groundbreaking research and biomedical innovations, but in recent years, has lost its edge in discoveries to other countries. This paper explores the inefficiencies of how the NIH allocates research funds, and then offers solutions to eliminate these issues. The paper also highlights how the 2020 global pandemic will affect research and clinical trials. The current state of the world affects how the NIH funds clinical research, and the future of this system may be altered due to the virus. The technical project will be carried out over the course of the 2020-2021 year within the Biomedical Engineering Capstone class. Capstone team members include Rebecca Della Croce, Zoe Garman, and myself. The technical advisor is Professor Richard Price from the Biomedical Engineering Department, with graduate mentors Catherine Gorick and Delaney Fisher.

DEVELOPING AN ASTROCYTE SIGNALING MODEL TO INFORM AND IMPROVE STROKE TREATMENT

The effects of the obstructed blood flow during stroke are vast and severe. Studies have found that over 50 percent of patients who survive beyond two days following their first stroke die within five years due to complications from the stroke (Hankey et al, 1998), including a subsequent stroke and heart disease (Bae et al, 2005). Treatment of stroke within four and a half hours of its onset is necessary to significantly reduce the risk of long-term disability and mortality; beyond that time, treatment efficacy decreases significantly (Musuka et al, 2015). Thus, given the high rates of stroke occurrence and the severity of outcomes, effective treatments are critical.

CURRENT THERAPIES AND MODELS

Due to the time-sensitive nature of ischemic stroke, current treatments are limited, and treatments focus predominantly on removing the obstruction. Therapies such as delivery of tissue plasminogen (tPA) activator are used to dissolve clots in order to improve blood flow and prevent ischemic damage (Baig et al, 2020). These treatments appear promising; however, many patient populations are not eligible due to restrictions (Hinkle et al, 2007). Additionally, tPA has only been found to prevent disability in six out of every 1000 strokes and also increases the risk of bleeding in the brain (Donnan et al, 2008). Mechanical devices are designed to retrieve and remove the occlusion. These tools have proven effective, but they are only used in approximately three percent of hospitals (Hameed et al, 2017). They are also only effective on the first pass in approximately one quarter of patients, despite first pass success being necessary for the best outcomes (Zaidat, 2018).

There are several existing computational models that relate to stroke, but they typically are not robust enough to be used to identify new therapies. Models have been developed to simulate recovery of motor function following stroke, but these models cannot be used to

identify specific treatment targets in the brain (Casadio et al, 2013, Colombo et al, 2011). Other models have been created to detail intracellular communication following stroke. However, these models depict a limited number of communication pathways, which limits the extent to which the model can be used to understand interactions within cells (Dronne et al, 2004).

EXPERIMENTAL HYPOTHESIS

This lack of an effective treatment and the knowledge gap that limits the development of new treatments have governed our modeling and hypothesis. We hypothesize that delivering mitochondria to astrocytes following stroke will increase the number of factors that promote recovery in the released exosomes.

Astrocytes are specialized glia cells and are the most abundant cell type in the central nervous system (Sofroniew et al, 2009). They play an essential role in maintaining normal brain function. Following an ischemic stroke, astrocytes carry out multiple functions that both benefit and damage neurons, making them an excellent therapeutic target to improve functions in the central nervous system (Liu et al, 2016).

Exosomes are extracellular vesicles that transport proteins, nucleic acids, and lipids between cells over long or short expanses and are proficient in manipulating target cells (Upadhya et al, 2020). Exosomes released by neural cells play an important role in communication between these cells and the periphery in both normal and disease (Upadhya et al, 2020). Being able to manipulate the phenotype of these exosomes is important for preventing further brain degradation post stroke and developing new treatments. Recent studies in ischemic heart disease have successfully shown that delivering mitochondria to the affected area promotes recoveries (Masuzawa et al, 2013). Ischemic stroke follows a similar mechanism to ischemic injury in the heart, and thus the impact of mitochondria delivery is also an area of interest in

ischemic stroke research. Mitochondria could aid in recovery from ischemic stroke, potentially by altering the biogenesis of exosomes in astrocytes such that they positively impact the surrounding cells.

EXPERIMENTAL DESIGN AND APPROACH

Throughout the year, we seek to construct our model to visualize astrocyte signaling pathways, apply this model to understand the connection between mitochondrial uptake and exosome release, and validate our results with cell culture experiments in both normal and stroke conditions, as seen in Figure 1. To construct our model in normal conditions, we will conduct an

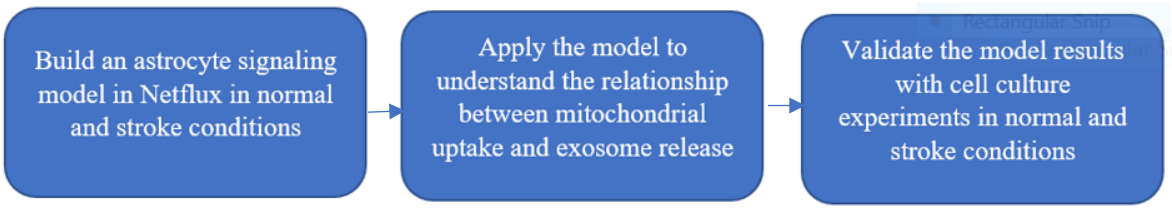

Figure 1: Experimental Approach. This figure explains the three specific aims of the technical design project. Building the model requires an extensive literature review and the use of Matlab's Netflux software. Applying the model requires manipulating the inputs in order to determine the mitochondria and exosome relationship. Validating the model includes in-lab experiments with astrocyte cell cultures, exosome harvesting, and model stroke conditions (Ford, 2020).

extensive literature review of known and common intracellular communications in normal astrocytes to have a complete understanding of astrocyte functions and interactions within the central nervous system (CNS). We will use the Netflux program in MATLAB to create our model by inputting variables and downstream targets discovered through our literature review. To build this model in stroke conditions, we will research the role of astrocytes in stroke conditions to see how this will alter our parameters within Netflux with the goal of providing an understanding of how they exacerbate or deter damaging effects in the CNS. To apply our model and test our hypothesis, we will adjust the model's input parameters to visualize how

mitochondrial uptake might impact exosome and neurotrophic factor release following stroke. We also hope to model the effects of focused ultrasound, a noninvasive therapy used to open the restrictive blood brain barrier for better treatment of CNS diseases. We will do so by adjusting input parameters to simulate the application of focused ultrasound based off of previous protein change data from our lab. Eventually, we hope to confirm our findings through cultured human astrocyte experiments. We plan to treat the cells with extracted mitochondria and then isolate the exosomes released from these cells after treatment. We hope our model will confirm our hypothesis that delivering mitochondria to astrocytes following stroke will increase the number of neurotrophic factors released in exosomes, and we also hope to publish these findings in a scientific journal to inform and improve current and future stroke treatments.

ETHICAL CONCERNS OF BIOMEDICAL RESEARCH FUNDING

The technical project could provide a method to treat strokes, which have devastating effects on American people. In order for the model to be validated, the hypothesis relies heavily on research before it could be implemented in medical care. According to Bluestone et al., the US has in recent years, lost its preeminence in biomedical research to other countries. Following an analysis made by the Journal of American Medical Association, it was concluded that if trends continue, Europe and Asia will surpass the US in leadership (Michaud, 2015). This decline threatens its ability to produce new therapies, as well could risk a business that provides over 300,000 jobs (Bluestone et al, 2018). The inefficiencies of the federal funding may be the cause and, if so, the "biomedical science system needs major reform" (Malakoff, 2014).

INEFFICIENCIES OF THE NATIONAL INSTITUTE OF HEALTH

While most research breakthroughs arise in academic-based or translational research funded by the government, only 22 percent of biomedical research funding comes from the federal government. The US federal funding trend has been on the decline with a 19.2 percent decrease from 2003 in 2017 (Bluestone et al., 2018). This decline has had two devastating effects, in which academic funding grants are not being accepted, and a decline in young people's faith in the NIH funding, decreasing the amount of young people entering the field. Additionally, one tenth of the NIH funds goes to their NIH employees. In recent years, the chance of breakthroughs originating from this intramural program has declined, due to unpredictable funding and a lack of attention to young, bold researchers, as shown by an independent study carried out by the NIH in 2014 (Bluestone et al., 2018).

This continued issue of NIH funding more traditional researchers has become a concern within the biomedical field. For example, it has long been accepted in research communities that Amyloid-beta is somehow responsible for Alzheimer's disease, while this has never been proven. Recently, it has become evident the suppression of other hypotheses, and lack of variable funding, has held Alzheimer's research back for years (Begley, 2019). This ethical concern must be amended in order to accelerate the US's ability to discover biomedical breakthroughs.

According to Sheth, research for cancer, stroke, and heart disease have all been paused or delayed by the lockdown caused in America due to the COVID-19 pandemic. Over 200,000 clinical trials across the United States were affected. Although the lockdown is necessary, the neglected disorders could likely cause a higher percentage of deaths, and clinical trials must resume (Sheth, 2020). The NIH must be altered to more ethically allocate funds in order to improve the US standing, but these alterations must consider the situation of the global pandemic.

ANALYSIS OF RELEVANT SOCIAL GROUPS

In order to successfully address the mentioned shortcomings of the NIH, it is important to consider the relevant stakeholders. The Social Construction of Technology (SCOT) (Bijker and Pinch, 1984) refers to the theory that explains how a variety of social factors shape a technological development, or a technological change (Johnson, 2010). According to SCOT, the technology refers to the NIH allocation system, or process, to research labs. The social groups that influence this system, and are in turn effected by the system, as seen in Figure 2, includes: NIH or government officials who decide the budget, NIH employees who review and allocate funds to research labs, researchers or

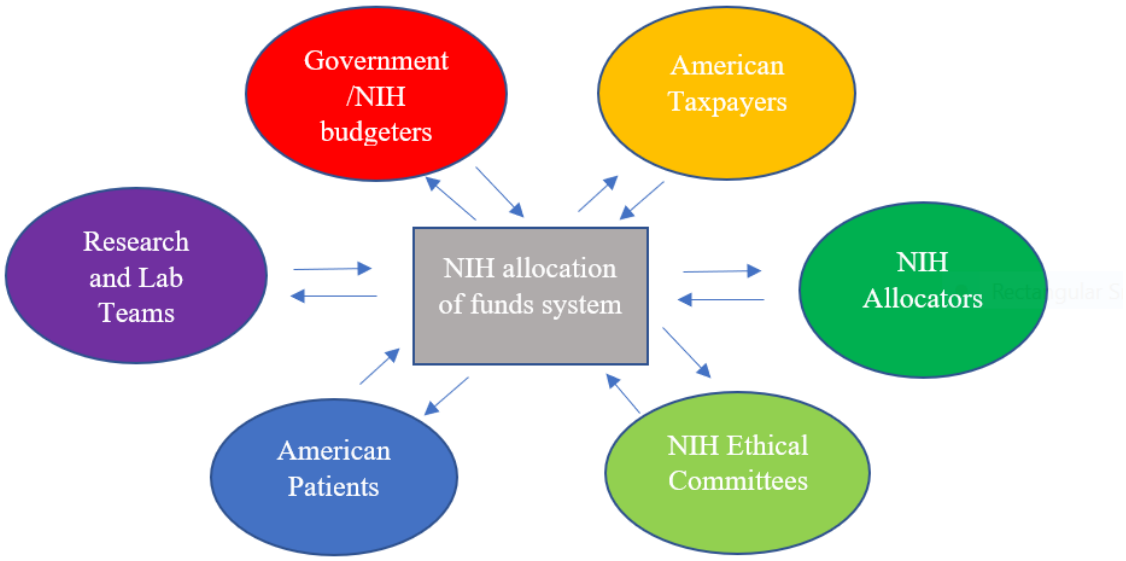

Figure 2: The Relevant Social Groups that Influence the NIH Fund Allocation System. This figure depicts how the six social groups, NIH or government officials who decide the budget, NIH employees who review and allocate funds to research labs, researchers or the lab teams themselves, ethical committees within the NIH that monitor how funds are used, American taxpayers, and the individuals throughout the world who benefit from the medical breakthroughs that result from the funding, influence the NIH system, and are directly affected by the system outcomes. The differing colors representing the distinct interests (Ford, 2020).

the lab teams themselves, ethical committees within the NIH, American taxpayers, and the individuals throughout the world who benefit from the medical breakthroughs. The government

must propose a budget for the NIH, with the interests of the taxpayers and America's standing in biomedical research in mind. The NIH employs experts to review grant applications and appropriately allocate the budget. Their interests could be bias, as they may desire to allocate more funds to research that backs their own findings, such as in the previously stated Alzheimer's example. Research labs rely heavily on consistent funds in order to pay for their experimental expenses. Institute Ethical Committees monitor the research that resulted from the

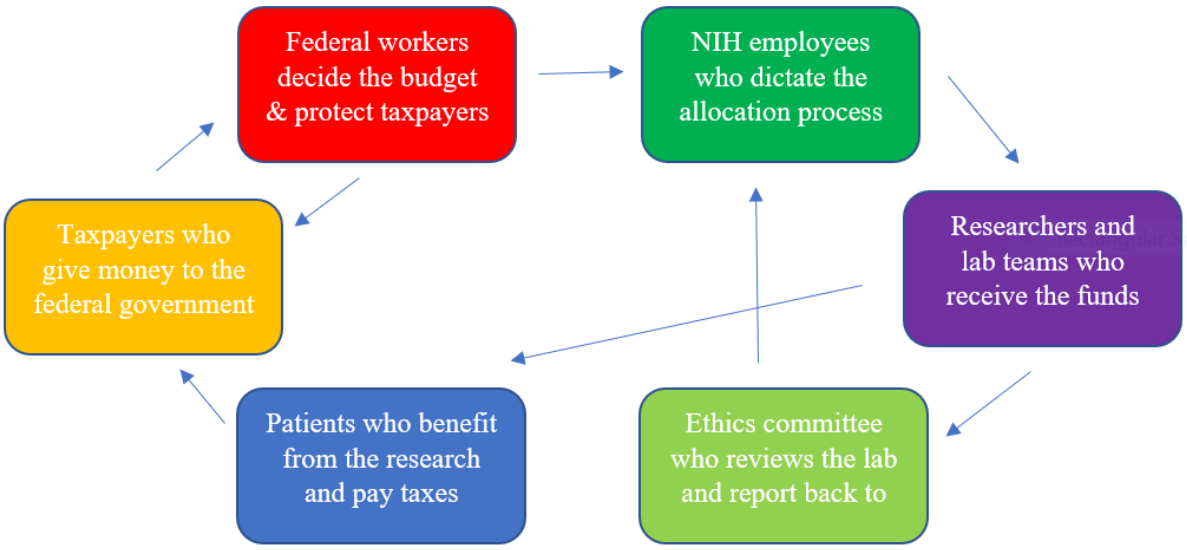

Figure 3: Revised Handoff Model. This revised handoff model shows how the relevant social groups not only influence the technology, but interact with other social groups (Ford, 2020).

funds, in order to make sure it was used ethically (Mandal et al, 2012). American taxpayers contribute the money to the NIH budget, and are interested in making sure their money is used properly. Lastly, individuals who suffer from diseases all greatly benefit from biomedical breakthroughs. All of these social groups influence how the system operates, and are directly affected by the system outcomes. Not only do they interact with the NIH allocation process, they also interact and influence each other, as seen in the revised handoff model depicted in Figure 3.

PROPOSED SOLUTIONS FOR THE NIH FOLLWOWING SCOT ANALYSIS

According to Mandal et al., “the responsibility for ensuring that the funds and resources are utilized optimally without any misconduct rests on the shoulders of the researchers, as well as the respective institute ethics committees along with the funding organizations” (Mandal et al., 2012). The first step in ensuring ethical codes are abided by is to provide more power to these groups. Their job is to be unbiased, so they should check and balance the power within the NIH.

The second recommendation considers the underrepresented portion of research labs promoting novel ideas and bold experiments. This section within the larger social group allows variability within the allocations, resulting in a greater chance of success. The intramural program structure should be reformed and NIH employees who review grants should be forced to consider smaller, new research groups. A quota should be met to delineate from the dominate theories. This ensures the taxpayers’ money is being used to improve American lives, not just to back certain scientists’ theories. It ensures the government is using the budget wisely and patients may now have a better chance of receiving successful therapy.

A third recommendation is a cultural change in perspective of biomedical research significance. Government leaders need to advocate for increased budgets for medical research. The people of America greatly benefit from breakthroughs, but it also improves the country's status globally. A better NIH budget provides jobs for hundreds of thousands of Americans, as well provides a business for pharmaceuticals, medical device companies, hospitals, and more. It is an important aspect of our economy, and should be given more attention by taxpayers.

Finally, the global pandemic has greatly shifted the focus of this issue. A great portion of the NIH budget now targets treating the COVID-19 virus. Although the NIH will benefit greatly from spending funds on research that will help to cure this virus, they must consider the impact

of neglecting other diseases. The NIH should continue to restructure how clinical trials will be carried out in a social distant, and safe manner. NIH employees should think creatively on how to continue important research, such as the use of wearable trackers in remote clinical trials.

AMERICAN BIOMEDICAL RESEARCH MOVING FORWARD

This paper only broadly addresses and suggests solutions for the issues plaguing the NIH. The purpose is to draw attention to these concerns in order to improve our biomedical field and return the US to its former prominence. These improvements will also help American public health and the country's economy. Ethical and economic problems must be addressed in order to improve the NIH's system of allocating research funds in biomedical research labs across the country.

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