The Importance of Acknowledgment in Increased Permeability of the Blood-Brain Barrier Under Chronic Immobilization Stress

Claire Margaret Shi

***Abstract*—The brain uses a large proportion of our daily energy intake despite its small mass percentage relative to our entire body. The system that transports this vital energy to the various structures in the brain, in the form of glucose, is composed of intricate blood vessels that remain isolated from the brain mass, thanks to the blood-brain barrier. But this seemingly impermeable armor of protection can be vulnerable to an emotion we are all familiar with – stress.**

Hyperpermeability of the blood-brain barrier is more common and dangerous than it might sound. The gag response experienced on the late Saturday nights after excessive alcohol consumption is one example of waste materials entering and endangering the brain. Alcohol, because of its unique chemical composition, easily penetrates the blood-brain barrier and disrupts the communication between neurons, causing reckless behavior and sluggish information perception when one is intoxicated. The gag response is our brain's attempt to protect the brain again foreign contaminants. So, the next time we chug down a mug of beer, perhaps take a moment to consider the invisible victim of our indulgences.

To test the hypothesis that chronic immobilization stress increases the permeability of the blood-brain barrier, two groups of researchers both used plastic tubes to restrict the activity of groups of mice for a different duration of days. At the end of their experiments, both groups injected the mice with chemical tracers. They examined the movement of the dye into the mice's brain to investigate whether more dye has leaked into the experimental group mice's brain relative to that of the control group, as an indication of disrupted blood-brain barrier function as a result of the immobilization treatment.

We are no stranger to the insomnia, migraines, and stomach aches artistically named "butterflies" when placed in extremely stressful environments. The disruption of the blood-brain barrier is a possible additional aliment induced by chronic stress. If conclusive evidence can be secured to prove the causational relationship between chronic confinement stress and a compromised blood-brain barrier function, doctors and hospitals would be able to prescribe more systematic treatments to patients displaying behavioral and neurological conditions associated with both psychological stress and blood-brain barrier breakdown.

***Index Terms*—Blood-brain barrier dysfunction, chronic immobilization stress.**

I. Introduction

The recent Covid-19 outbreak has led to emotional stress and anxiety in both doctors and civilians [1]. Under such circumstances, it becomes more important to examine "stress" on a medical level. Research has proven that chronic stress can compromise our immune system and cause diseases such as chronic hypertension, arrythmia, and left ventricular hypertrophy [2]. But even more, recent studies have also suggested that chronic stress takes a toll on the brain and can potentially disrupt the permeability of the blood-brain barrier – the system of isolation composed of specialized endothelial cells and tight junctions that restricts the interaction between energy-suppling blood vessels travelling through the brain and our neurons, preventing the entry of bacteria, pathogens, and other foreign substances into the brain [3]. Chronic stress has been found to alter the expression and structure of proteins in these tight junctions, thus disrupting the normal functions of the blood-brain barrier and increasing its permeability [3]. The blood-brain barrier has a critical role in maintaining the sanitation of the brain, making sure that it is not contaminated by foreign substances. Blood-brain barrier dysfunction, the breakdown of the entire system of cells, can lead to various neurological diseases, including multiple sclerosis, stroke, and epilepsy [4]. The disruption of the blood-brain barrier is also associated with major psychiatric disorders such as depression and schizophrenia. Researchers have even hypothesized that blood-brain barrier dysfunction can be an "early indicator of Alzheimer's disease" [5].

The purpose of this literary review is to consider the merit of the argument that immobilization stress – the restriction of movement, also referred to as confinement stress (this paper would use these two terms interchangeably) – can damage the blood-brain barrier and increase its permeability. This paper will present experiments in which researchers test this idea and highlight the procedures. This paper will also offer possible reasons for the discrepancies seen between the final results of the two experiments. Supporting evidence and supplementary data will also be included to support the additional findings introduced by the researchers.

II. Literature Review

A. Introduction

The scientific community has established many research pieces to test the relationship between chronic stress and a compromised blood-brain barrier. While there is evidence that supports this hypothesis, there are also other data that suggest there are other confounding variables that undermine the significance of these final results.

B. Evidence Supporting Increased Permeability in the Blood-Brain Barrier due to Chronic Immobilization Stress

A team of Chinese researchers uncovered results that

Manuscript received November 12, 2021; revised February 10, 2022.

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doi:10.18178/ijssh.2022.12.2.1067

support the hypothesis, using several experimental groups of mice, each under the same confinement stress treatments for 6 hours a day for 1, 3, 7, 14, and 21 days. The researchers randomly assigned the mice to six groups – five experimental groups that underwent immobilization stress and one control group used as a baseline comparison. The control group was handled 5 minutes to transfer cages and fasted for 6 hours each day, simulating the experimental groups' environment during the restriction period using plastic tubes. The handling and fasting procedures the control group experienced ensures that the final differences in the permeability of the blood-brain barrier between the experimental and control groups will be only due to the confinement treatment instead of any other environmental factors. At the end of the experiment, the researchers injected all mice with Evans Blue dye tracer. They then measured the amount of dye that leaked into the brain after two hours of circulation time -- the time between the injecting the tracer into the mouse and dissecting the brain for examination. After dissection, the researchers concluded that the permeability of the blood-brain barrier in the amygdala of experimental group mice (those in the 14 and 21-day groups) increased compared to the control group. These results provide sufficient evidence to suggest a relationship between confinement stress and compromised blood-brain barrier function. The selectivity in the experimental groups displaying increased permeability in the blood-brain barrier also showed that only groups under persistent chronic stress for long periods, 14 and 21 days, exhibited increased permeability in the blood-brain barrier, reflecting the importance of the duration of stress that can impact the blood-brain barrier [6].

C. Evidence Rejecting Increased Permeability in the Blood-Brain Barrier due to Chronic Immobilization Stress

However, other data also seem to contradict these results, such as a similar experiment conducted by Martin Roszkowski and Johannes Bohacek. The experimental group received the same treatment of immobilization stress with plastic tubes of 6 hours for 21 days. The environmental factors were all held constant, and the control group was also transferred to different cages to simulate the transferring process the experimental group underwent. On day 22, the mice were injected with a chemical tracer of NaF and given 10 minutes for the tracer to circulate and enter the subjects' brain. The final results contradicted the former experiment: Mice in the experimental group showed no significant increase in blood-brain barrier permeability than the control group. Thus, the researchers concluded that immobilization stress has no significant effect on the permeability of the blood-brain barrier [7].

III. Discussion

A. Possible Explanations for Difference Between the Conclusions Reached by the Two Experiments

There are several possible explanations for these discrepancies. Roszkowski and Bohacek focused on the hippocampus, cortex, and cerebellum, while the Chinese researchers focused on the amygdala. The researchers examined different sections of the brain, and therefore these results are not mutually exclusive and can coexist. Further research localizing the effects of chronic confinement stress on the blood vessels' permeability in individual areas of the brain might elucidate whether the blood-brain barrier in different brain structures respond differently to chronic confinement stress.

A second possible reason might be the short circulation time allowed in Roszkowski and Bohacek's experiment. The Chinese researchers gave the mice 2 hours for the tracer to circulate through the body and diffuse into the brain when Roszkowski and Bohacek allowed only 10 minutes. While one complete circulation of blood through a mouse only takes 15 seconds [8], the blood-brain barrier potentially delays the passage of macromolecules into the brain, indicating that a longer circulation time is needed for the tracer to sufficiently enter the brain and accurately reflect the permeability of the blood-brain barrier before and after the confinement treatment. By examining the bar graphs, we can see that there is indeed a slight decrease in mean CER, or cerebral extraction ratio, between the control group and the restraint group, suggesting a disruption in the blood-brain barrier is plausible, though not yet significant enough to suggest a relationship between the treatments and the final observed data because of their overlapping error bars.

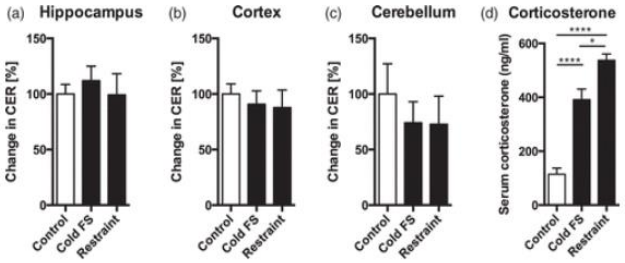

Fig. 1. Data provided by Roszkowski and Bohacek showing the percent change in CER comparing the control group and the other two experimental groups.

With a longer circulation time, the percentage CER change might create a statistically significant difference between the results of the experimental group and the control group and show more clearly a disruption in the blood-brain barrier. Roszkowski and Bohacek did design two groups of non-stressed mice with different circulation times, one with 10 minutes and the other with 30 minutes. The results showed that the group given longer circulation time (30 minutes) indeed had a more significant CER change than the group given 10 minutes of circulation time, suggesting that longer circulation times can possibility amplify the differences between the percentage CER change.

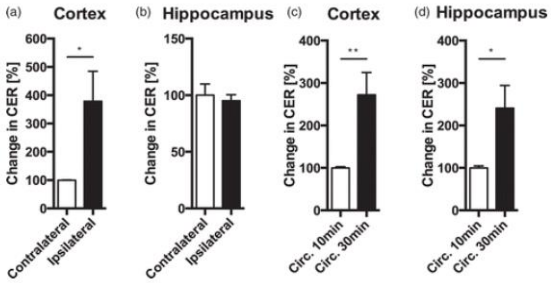

Fig. 2. Data provided by Roszkowski and Bohacek comparing the percent change in CER of the 10-minute circulation time group and 30-minute circulation time group of non-stressed mice.

B. Need for Further Inquiry

Other questions also remain regarding the relationship between chronic stress and elevated permeability of the blood-brain barrier. The time needed for macromolecules to enter the brain with a completely functional blood-brain barrier is a factor that we need to consider when designing experiments and examining data to decide whether the experimental design gives enough time for chemical tracers to penetrate the blood-brain barrier and reveal visible changes in the permeability of the blood-brain barrier.

The data from Roszkowski and Bohacek's experiment also proposes that temperature is a possible confounding variable, that hypothermia disrupted the blood-brain barrier more than stress.

Both hypothermia experiments, cold forced swims (Cold FS) and cold restraint, had experimental groups that yielded results significantly deviating from that of the control group. With these data, the researchers provide sufficient evidence to show that low temperatures can disrupt the blood-brain barrier, making this a possible path for further inquiry.

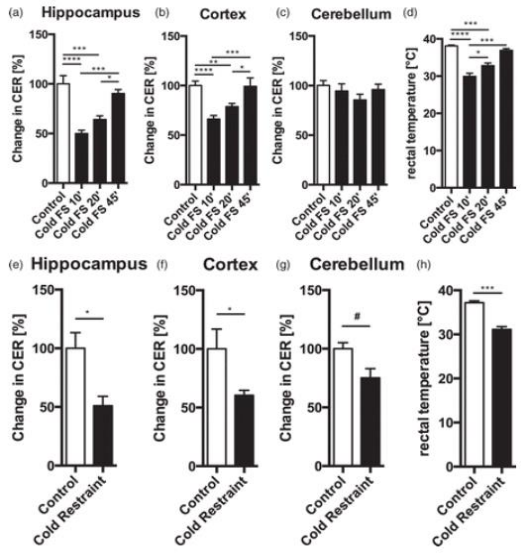

Fig. 3. Data provided by Roszkowski and Bohacek showing the percent change in CER in different sections of the brain after different durations of hypothermia, either in the form of forced cold swims or immobilization stress under low temperatures.

Most recent research regarding the effects of hypothermia on the permeability of the blood brain barrier agree that hypothermia can alleviate symptoms of blood-brain barrier dysfunction in subjects who have suffered from brain trauma. Less experiments investigate the effect of extreme low temperatures on healthy brains. Researchers such as Kiyakin and Sharma have observed that brain temperatures can influence the permeability of the blood brain barrier, specifically where extreme hypothermia at temperatures 32℃-33℃ is associated with elevated blood-brain barrier leakage in experimental subjects compared to the control group [9]. Roszkowski and Bohacek's hypothermia environments, however, were set at 18℃, far colder than the conditions in Kiyakin and Sharma's experiments, which possible exasperated blood-brain barrier leakage induced by the extremely cold environments and leading to more obvious results. Further experimentation containing various experimental groups situated in different temperatures (ideally ranging from hypothermia to normal to hyperthermia) will provide more insight into the influence brain temperature has on blood-brain barrier permeability.

C. Applicational Values

The applicational worth of these data need to be thoroughly examined. Experiments had not yet been performed with other types of stressors to provide sufficient evidence to support a similar relationship between a compromised blood-brain barrier and the various types of other stressors that are more common to our daily life, such as academic and socioeconomic pressure. Additionally, we have not yet performed similar experiments on human subjects; thus, researchers still need to determine whether humans exhibit similar changes in blood-brain barrier functions in response to chronic confinement stress. We will need to conduct additional research to visualize the impact blood-brain barrier dysfunction has on the individual experiment participant.

IV. Conclusion

The Covid-19 outbreak had now claimed the lives of nearly 6 million people worldwide [10]. More souls were lost to suicide due to economic recessions, fear of infections, and unemployment [1]. Under this situation, managing our stress and anxiety is becoming essential to maintaining our health and is attracting more public attention as we start to understand mental disorders. Researches provide data to suggest a causative relationship between chronic immobilization stress and disruption in the blood-brain barrier. While different experiments have reached diverse conclusions to explain the disruption of the blood-brain barrier observed, better-designed experiments and studies uncovering the relationship between the permeability of the blood-brain barrier and other confounding variables would shed light onto the details of the issue. This information has the potential to revolutionize the field of pharmaceuticals, allowing future medical professionals to connect stressful lifestyles with behavioral and neurological diseases associated with blood-brain barrier dysfunctions. If this causational relationship between stress and blood-brain barrier break down can be validated, doctors would have more solutions for patients with these illnesses, such as prescribing psychological counseling and medications to alleviate the patient's stress, indirectly handling the conditions. New treatments would provide more angles from which we can cure these diseases, creating holistic and associable medical plans for the many suffering from these illnesses.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Paper written by Claire Margaret Shi. All authors have approved the publication of this paper.

Acknowledgment

Claire Margaret Shi expresses her sincere gratitude to

Professor Eric H. Schumacher and mentors involved in helping with the editing and formatting of this paper's content. Shi participated in a Psychology program focused on the physical manifestations of stress and happiness, led by Professor Eric H. Schumacher from the Georgia Institute of Technology. Inspired by the course, Shi was able to construct this paper based on the knowledge acquired in the program.

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