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The Role of Acupuncture in Accelerating the Patients' Recovery and Improving the Patients' Long-Term Prognosis

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Abstract

Acupuncture is an integral component of traditional medicine in Asia and has been gaining global attention for its possible analgesic sparing effects. Several studies have shown that acupuncture may improve post anesthetic recovery by reducing anxiety, pain, dosage of anesthesia required, and physiological complications that follow operations. Thus, acupuncture can have clinical significance in pain management and post-surgery recovery. However, the underlying mechanism of acupuncture that explains its physiological effects remains poorly understood. This paper aims to examine and evaluate previous research that studies the effectiveness of acupuncture in management of postoperative complications—including acupuncture's possible role in reducing preoperative anxiety, reducing the dose of anesthetics in operations, preventing physiological and cognitive dysfunctions, and accelerating recovery—across various cases of surgeries. In addition, this paper calls for a more comprehensive way to study the molecular mechanisms behind acupuncture that alter the molecular profiling of patients and ultimately exert its effect on their prognosis.

Keywords: Analgesic, Anesthesia, Pain management, Post-surgery recover, Molecular profiling

1. Introduction

Acupuncture is generally known to have originated in China, but even in ancient China around 6000 BCE, it was just a surgical tool to draw blood and incise the abscess in the form of sharp stones and bones (White & Ernst, 2004). For several thousands of years, acupuncture has been a traditional remedy from generation to generation just like herbs, massage, diet and moxibustion, burning dried mugwort on the body. In about 100 BCE, an organized diagnostic and treatment system recognized as acupuncture is first mentioned and described in The Yellow Emperor's Classic of Internal Medicine (White & Ernst, 2004). Since then, not only Chinese acupuncture developed exponentially, it has spread to other countries as well, because information and knowledge about acupuncture could be documented and delivered more efficiently to future generations. Within about 7 centuries, acupuncture has spread to nearby Korea and Japan while it was not generally accepted until 19th century in Europe. For thousands of years since then, acupuncture, an integral part of traditional Chinese medicine, has attracted great interest in the Western world. It focuses on stimulating certain points on the body with needles to enhance circulation and neurological functions (Taguchi, et

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al., 2002). Researchers have taken interest in acupuncture's possible role in managing pain and promoting post anesthetic recovery (Fleckenstein, et al., 2014). Acupuncture in its philosophic meaning is not primarily supposed to 'cure' illness - the underlying idea is that acupuncture may harmonise *Qi*, which is a Chinese concept of vitality or energy, and the alleviation of respective symptoms (Fleckenstein, et al., 2014). Usage with anesthesia, acupuncture would have positive effects on pre- and postoperative care. This review aims to examine and evaluate results from different studies that focus on acupuncture's effects on the patients before and after surgery.

2. Acupuncture could significantly reduce preoperative anxiety and dosage of narcotic drugs

A meta-analysis of randomized controlled trials (RCTs) studying the efficacy of acupuncture in reducing preoperative anxiety demonstrates that acupuncture significantly reduces anxiety prior to surgery as compared to control (no treatment) and placebo conditions (Bae, et al., 2014). Patients undergoing cardiac surgery exhibited lower preoperative anxiety and achieved sedation more quickly when given acupoints stimulation 30 minutes prior to anesthesia (Shan, et al., 2010). Similarly, another study demonstrated that acupuncture reduces the required dose of a sedative drug in critically ill patients (Zheng, et al., 2012). In this study, a group of patients given electrical stimulation at certain acupuncture points for 6 hours achieved sedation at a significantly lower dose of sedative drug (midazolam) compared to a control group given no acupuncture treatment. Moreover, a study conducted on patients undergoing cataract surgery showed that the group given acupuncture 20 minutes before the surgery had significantly reduced preoperative anxiety levels, as measured by the Visual Analog Scale, compared to the control group given no treatment and the group given a sham treatment (Gioia, et al., 2006). Thus, studies support the role of acupuncture in effectively reducing anxiety related to surgery.

Several studies have shown that acupoint stimulation lowers the dose of intraoperative anesthetics. One study examined patients undergoing open heart surgery in which one group of patients received anesthesia in combination with acupuncture and the other group received conventional anesthesia alone. In this study, researchers concluded that compared to the group receiving conventional anesthesia alone, the group receiving the combined acupuncture-anesthesia required less usage of narcotic drugs, fentanyl and midazolam (Zhou, et al., 2011). In fact, the combined acupuncture-anesthesia treatment reduced the required dose of anesthetic by 10%~50%. Similarly, a study that examined 80 cases of heart valve replacement surgery also observed a significant reduction of dosage of anesthetics (Fentanyl, Midazolam, Vecuronium Bromide) needed by a group of patients that received electroacupuncture stimulation as compared to the control group that did not receive electroacupuncture stimulation (Chi, et al., 2014).

3. Acupuncture may function to protect intraoperative functions of vital organs

Treatment of patients with electric acupuncture prior to surgery has shown to stabilize their intraoperative respiratory and circulatory functions. In one particular study, a group of patients given electric acupuncture 30 minutes prior to pneumonectomy showed significantly less fluctuations in the mean arterial pressure and heart rate than those in the control group (Ma, et al., 2011). In this study, the group given electric acupuncture also demonstrated less increase in plasma stress hormones, adrenaline and cortisol, before and after the operation, indicating acupuncture's possible role in the reduction of stress responses (Ma, et al., 2011). In another study that examined patients undergoing craniotomy, the group of patients given electric acupuncture exhibited more stable heart rates and arterial pressure during the surgery compared to the control group (Wu, et al., 2013). Moreover, the levels of stress hormones including cortisol, epinephrine, and blood glucose, were significantly lower in the group given electric acupuncture (Wu, et al., 2013). Similarly, another study of patients undergoing thyroidectomy found that electric acupuncture

stabilized the mean arterial pressure and reduced the amount of propofol used during the surgery (Yan, et al., 2014). Furthermore, one study of patients undergoing heart valve replacement examined the level of oxidative stress by measuring the serum levels of malondialdehyde (MDA), cardiac troponin I (cTnI), and superoxide dismutase (SOD). In this study, the group of patients given electric acupuncture had significantly reduced serum levels of MDA and cTnI and increased levels of SOD after the surgery, which support that this group experienced lower oxidative stress compared to the control (Ma, et al., 2015).

A troponin test measures the serum level of troponin I, which are proteins released when there is damage to the heart muscle. Thus, the cTnI level is indicative of heart damage. Researchers have found that the cTnI level was significantly lower for patients who were given electroacupuncture prior to percutaneous coronary intervention than for patients that were not given the electroacupuncture treatment (Wang, et al., 2015). Similar results were observed with patients undergoing heart valve replacement. In this study, the group given electroacupuncture demonstrated significantly reduced cTnI level once the aorta was opened (Yang, et al., 2009). Furthermore, patients exhibited improvement in microcirculation of brain tissue, as evidenced by regulated levels of plasma endothelin and calcitonin gene-related peptide, when given electroacupuncture treatment prior to craniotomy (Wang, et al., 2008). These patients also exhibited lower levels of interleukin-6, hence decreased proinflammatory response. Thus, electroacupuncture could have a protective effect on vital organs including the heart and the brain.

4. Acupuncture shortens extubation time and postoperative complications

Patients given acupuncture demonstrated faster recovery time as evidenced by shorter postoperative extubation and memory recovery time. In one study of patients undergoing sinusotomies, the acupuncture group had shorter postoperative extubation times (12.5 ± 3.5 vs. 17.3 ± 6.7 min) and shorter memory recovery time (16.4 ± 5.9 min vs. 21.8 ± 8.7 min) compared to the control group (Wang, et al., 2014). In another case of patients undergoing ambulatory breast surgery, patients that received electric acupoint stimulation had a significantly shorter lengths of recovery room stay (35.6 ± 12.9 min vs. 48.3 ± 16.3 min), shorter time for the removal of the laryngeal mask (10.2 ± 2.5 min vs. 17.8 ± 4.4 min), and time to reorientation of the patient (14.6 ± 3.2 min vs. 26.5 ± 5 min) (Zhang, et al., 2014). In addition to shortened extubation and memory recovery time, patients with acupuncture treatment had shorter extubation to the 'ready for discharge' period than patients without acupuncture treatment. One randomized controlled trial examined the effect of acupuncture on post-operative recovery (Fleckenstein, et al., 2018). Seventy-five patients undergoing gynecologic laparoscopy that involved anesthesia were randomly assigned to receive either acupuncture treatment, sham treatment, or no treatment. Researchers found the group that received acupuncture treatment demonstrated significantly reduced extubation to the 'ready for discharge' period (median 30 (24-41) min) compared to the control group that received no treatment (46 median (36 to 64 range) min) and to the group that received sham treatment (43 median (31 to 58 range) min) (Fleckenstein, et al., 2018). They concluded that the median time to reach the ready for discharge point in the group that received acupuncture was 35% less than in the control group and 29% less than in the group that received sham treatment. This difference in time to recovery was considered clinically relevant (Fleckenstein, et al., 2018). Therefore, acupuncture could shorten the recovery time as evidenced by the reduced time to extubation and from extubation to 'ready for discharge' point.

Pain control post-surgery is critical for proper recovery since lack of proper and complete pain control can lead to immunosuppression, which could further complicate wound healing, prolong recovery, and increase the risk of postoperative infection (Yuan and Wang, 2019). Moreover, insufficient pain control can lead to psychological complications including anxiety and depression (Yuan and Wang, 2019). Inadequate pain control post-surgery can become chronic, so effective pain management post-surgery is crucial for the patients' recovery and health. Studies

have shown that acupoint stimulation can relieve pain post-surgery. In one study, patients undergoing elective gynecological laparoscopic surgery that were given the acupoint stimulation 30 minutes prior to anesthesia reported significantly lower postoperative abdominal pain score than those who were not given the acupoint stimulation (Yao, et al., 2015). A meta-analysis, evaluating the effectiveness of acupuncture in controlling post-operative pain, supported that the group that was given transcutaneous electric acupoint stimulation required significantly lower amount of opioid analgesic post-surgery than the control group did (Wu, et al., 2016). In addition to relieving pain, acupuncture has shown to prevent postoperative nausea and vomiting, which delays the recovery of patients. Cases in which postoperative nausea and vomiting were significantly reduced with electroacupuncture include gynecological laparoscopic surgery (Ertas, et al., 2015), breast surgery (Zhang, et al., 2014), and caesarian section (Liu, et al., 2015). Furthermore, acupuncture could have a protective role in cognitive functions post-surgery. A model of myocardial ischemia-reperfusion injury utilized on aged rats revealed that electroacupuncture could prevent postoperative cognitive dysfunction induced by the injury, as evidenced by reduced neuronal apoptosis and reduced levels of oxidative stress and inflammation (Yuan, et al., 2014). Another study utilized the water maze test to evaluate the rats' cognitive functions before and after intravenous propofol anesthesia. Here, they found that rats given electroacupuncture performed better after the anesthesia compared to the control rats (Liu, et al., 2016). In addition, they found that the group of rats given electroacupuncture had decreased GSK-3 phosphorylation level in the hippocampal CA1 region, suggesting that electroacupuncture could relieve some cognitive impairments via some opioid receptor-independent mechanism (Liu, et al., 2016). Another study used behavioral tests to observe cognitive dysfunctions after left lateral lobectomy. In such study, rats given electroacupuncture exhibited significantly improved cognitive abilities compared to the control and demonstrated significantly lower levels of proinflammatory cytokines in the hippocampus (Feng, et al., 2017). Randomized and controlled clinical studies showed similar results. Patients who were given electroacupuncture 30 minutes before anesthesia exhibited significantly better postoperative cognitive functions than control after a spinal surgery (Zhang, et al., 2017) and an abdominal surgery (Lin, et al., 2013).

Patients often experience immune dysfunctions following the surgery, so the recovery of postoperative immune function is critical to their long-term prognosis. Studies have shown that electroacupuncture in the case of breast surgery downregulates the level of proinflammatory cytokines including TNF-α while upregulating the level of anti-inflammatory cytokines including IL-2 and IL-10 (Zhang, et al., 2014). Similar results were observed with colorectal cancer patients. Patients who were given electroacupuncture 30 minutes after general anesthesia exhibited significantly lower levels of proinflammatory cytokines including TNF-α, IL-1β, and IL-6 post-surgery than patients that were not given electroacupuncture (Lin, et al., 2013). Such a profile reveals that acupuncture could improve immunosuppression from anesthesia.

5. Discussion

Categorizing the preoperative, intraoperative, and postoperative effects of acupuncture, acupuncture serves to reduce anxiety and dosage of narcotic drugs prior to operation. Acupuncture has intraoperative functions to reduce the fluctuation in patients' respiratory and circulatory functions, especially those undergoing thyroidectomy; also, acupuncture reduced intraoperative damage on heart muscles observed by decrease in cTnI levels. However, acupuncture must be practiced in the optimal depth and needle grasp in order to have the right pullout force to be effective.

While studies support acupuncture's protective and rehabilitative role pre- and post-operations, the exact underlying mechanism remains unclear. Thus, it is critical to study the cellular and molecular mechanisms that explain the phenomena described above. As mentioned previously, researchers Zhang, Lin, and Wang found some cellular and molecular changes that accompany acupuncture treatments. Yet, they fall short in just examining correlations and not causations. For example, previous researches

demonstrated that there is a decrease in the level of proinflammatory cytokines when patients were treated by electroacupuncture prior to surgery. However, this is just a correlation and not a causation. Further steps need to be taken to examine how exactly these proinflammatory cytokines may be affected by electroacupuncture and through which mechanism electroacupuncture can alter the molecular profiling of patients and eventually exert its effect on their prognosis.

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A Comparison of Direct Reversal and Nucleotide Excision Repair in UVB-Treated Bacteria

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Abstract

DNA of living organisms are sensitively being targeted by Ultraviolet radiation, and especially UVC and UVB are bringing damage to the DNA. Fortunately, living organisms have repairing mechanisms to maintain genetic diversity and be able to reproduce. This experiment compares the effectiveness of two repairing mechanisms of DNA, direct reversal repair and nucleotide excision repair. The findings of this research are relevant to the UV radiation treatment technologies, their advantages and disadvantages, and the possible improvement of them. In the experiment, bacteria collected from school's public bathroom space were used as a source of target DNA for UVB damages. Bacteria cultures were irritated under the UVB reptile lamp, after which the cultures were treated either with sunlight or in the dark. Treatment with sunlight represents the direct reversal repair, and the dark treatment represents nucleotide excision repair. These bacteria are then incubated at the temperature ideal for specific bacteria, E. coli to grow. Bacteria colonies were counted as result after 48 hours of incubation. The result of the research indicated that the direct reversal repair is more effective than nucleotide excision repair in the given circumstances. Energy efficiency differences of the two repairing mechanisms was provided as one of the explanations for this outcome. Unexpected results occurred that differed significantly from the rest of the results. For example, the bacteria culture that was not altered to UVB light seemed to be harmed by sunlight. These exceptions have been considered and their causes carefully explained.

Keywords:DNA repair mechanisms, UV radiations, Bacterial DNA, Nucleotide Excision Repair, Direct Reversal Repair

1. Introduction

This investigation is an attempt to research the repairing potential of bacteria after their DNA has been harmed by UVB-radiation. The study aims to answer the following question: Is direct reversal repair more effective than nucleotide excision repair on repairing the damaged DNA of bacteria E. coli, caused by various time intervals of UVB radiation, and determined by the numbers of bacteria colonies? The hypothesis is that the direct reversal repair using direct sunlight emission as a source of UVA radiation for photoreactivation of protein photolyase is more effective in repairing the damaged DNA of bacteria E. coli caused by UVB radiation, than nucleotide excision repair set in the dark.

While the repairing mechanisms are desirable for the survival for organisms, if not treated with

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care, it might cause detrimental troubles to human health. UV radiation treatment is used in the field of medicine to disinfect the surgical instruments before using them again, and the UV radiation in water treatment technology has also increased. UV-irritated microorganisms might have opportunity to repair and potentially reproduced during the time of retainment. This study also aims to examine whether the repair under sunlight or in the dark is of a greater concern.

1.1 Background Information

The sun emits three types of Ultraviolet Radiation (UV) which are UVA, UVB, and UVC, which differ from each other with their specific wavelengths and energy. UVC has a wavelength of 100 nm-280 nm, UVB 280 nm-315 nm and UVA 315 nm-400 nm. UVC is a short-wave radiation with high energy and seriously harmful for living organisms. Fortunately, the ozone layer located above the surface area of the earth absorbs 97-99% of the UVC (Gleason, 2008). The rest of the Ultraviolet radiation, UVA and UVB reach our skin. Approximately 95% of the UV light reaching the earth's surface is the long-wavelength UVA, while only about 5% is UVB radiation (WHO, 2016). UVB is more harmful of these two common UV lights, because it has enough energy to cause photochemical damage to cellular DNA (Zeman, 2013).

DNA of living organisms is especially strong at absorbing UVB radiations, and so it is an important target for UVB induced damage. According to (Salkinoja-Salonen, 2001, ss. p. 303-307) ''a variety of chemicals and environmental conditions increase the frequency of spontaneous mutation, such as high temperature, viruses, and UV radiation.'' High energy of UVB radiation alters a chemical bond in the thymine, causing crosslinking between adjacent bases of cytosine and thymine in DNA to mainly produce cyclobutene pyrimidine dimers (CPDs) (Salkinoja-Salonen, 2001). These pyrimidine dimers cause DNA strand to change its three-dimensional shape and is failed to be read and copied in the processes of transcription and translation. If a cell accumulates too many dimers, it might die or become cancerous (Budden & Bowden, 2013).

However, these organisms have repairing mechanisms that maintain their genetic diversity and enable them to reproduce and pass on those genes. Tomas Lindahl, Paul L. Modrich and Aziz Sancar were awarded The Nobel Prize for having discovered how cells repair their DNA. There are three main types of repairing mechanisms that are known: homologous recombination, direct reversal, and excision repair (Chang, 2016). In excision repair, a specific array of distortion on the double helix of DNA caused by mismatched base pairs are recognized by endonuclease and removed. Excision repair consists of Base excision repair (BER) and Nucleotide excision repair (NER) (Reardon & Sancar, 2006). The difference between NER and BER is that BER is a simple repairing mechanism that works to repair the DNA damages caused endogenously**.** NER, however, is a complex repair system that works in the cells to repair comparatively bigger, damaged regions caused exogenously, such as environmental and chemical carcinogens (Augusto-Pinto, et al., 2003). This experiment therefore concentrates on the effectiveness of NER because the damaging factor is UVB-radiation and exogenous. Neither of the excision repair requires light to take place (thus are also known as dark repair), therefore dark condition is provided for NER to take place in the experiment.

Currently, the third type of repair, direct reversal of the damage, is by far the simplest repair mechanism. One of the two conditions of the research, sunlight exposure - as a source of blue-light photons (300–500 nm) needed in the process of photoreactivation - represents the condition provided for direct reversal repair to take place after the UVB-radiation. A major polypeptide involved in this pathway is a protein called DNA photolyase, which is a light-driven enzyme responsible for removing cyclobutene pyrimidine dimers from DNA in a light-dependent process denominated photoreactivation (Essen & Klar, 2006). The damaged part of the DNA is recognized by this enzyme that absorbs the photons of the appropriate wavelength. This photon absorption enables the photochemical reactions to occur, and the pyrimidine dimers are removed (Sancar, 2003).

Because of its relatively quick reproduction in a relatively short amount of time thus showing visible repairing results, and among the more ethical choice

of altering living organisms to intentional harm, this experiment used bacteria as a source of life and DNA. As a real-life application example of the study, in the field of medicine, storing the surgical instruments after disinfection treatment might be a chance for repairing mechanisms to take place and can occur in bacteria-caused infections.

The blood agar petri dishes used in this experiment were an ideal condition for the growth of *E. coli*, which were recognized as yellowish colonies after incubation. Bacteria were treated as potential pathogens: its pathogenicity increases when the ideal conditions for them to grow and reproduce are provided. The natural habitat for *E. coli* is for example intestine, where the temperature is about 37 Celsius degrees. If such a temperature was kept for the bacteria culture, the growth would be dangerously quick and therefore the temperature was kept at 27 Celsius degrees. The bacteria cultures were handled with care and destroyed after use according to Finnish regulations.

1.2 Variables

There are two independent variables in this investigation. Different durations of UVB lamp exposure of bacteria cultures provided the experiment with six different settings, whose aim was to damage the DNA of bacteria in different scales. Sunlight exposure of 6/12 petri dishes to sunlight and 6/12 put in the darkness aimed to investigate the hypothesis, whether photo repair (direct reversal) is more effective that the dark repair (nucleotide excision). Dependent variable was the number of bacteria colonies after UVB treatment and sunlight exposure. Various control variables were also taken into consideration. To provide all the bacteria with the same temperature and condition, they were all kept next to the window, regardless of being covered or uncovered with aluminum foil. All petri dishes were also kept in the same incubator where the temperature was kept on 27 Celsius degrees for 48 hours. For avoiding the UVB-lamp intensity difference, the lamp was turned on 5 minutes before the first exposure, so that the possible ‘’warm-up phase’’ of the lamp will not have effect on the result; The UVB box is set up so that each petri dish was equally distant from the lamp (10 centimeters) and therefore equal intensity of 4680 Lux. Because sunlight was one of the independent variables that will affect the result, for keeping the same sunlight intensity the petri dishes were kept close to each other in the same place next to the window under the direct sunlight. Duration of sunlight exposure also needed to be equal for all bacteria cultures. All the petri dishes are kept under the sunlight for 4 hours, as it was performed in a similar previous study (Zimmer, 2002).

1.3 Hypothesis

The direct reversal repair using direct sunlight emission as a source of UVA radiation for photoreactivation of protein photolyase is more effective in repairing the damaged DNA of bacteria E. coli caused by UVB radiation, than nucleotide excision repair set in the dark. The average number of bacteria colonies will be more in the dishes of sunlight exposure than the ones set in the dark.

This hypothesis is based on multiple other works (Britt, 1995) about photolyase being a photon driven enzyme that is readily activated by certain wavelengths of light, and 130 that the direct reversal being the most efficient repairing mechanism, where photolyase is involved.

2. Materials and Methods

Many factors were considered while planning the method of the experiment to collect as precise and accurate data as possible for answering the research question. Firstly, the most suitable bacteria concentration for the experiment was discovered by testing three concentrations on the same petri dish before the actual experiment. The best concentration was found out to be 10^{-6}, where the bacteria colonies were not too much to be counted with eyes, and not too less to see whether the independent variables have affected their growth.

Secondly, the duration, exposure intensity and distance of petri dishes to UVB lamp were closely controlled. UVB irradiation treatment was performed with a High Output Reptile UVB200™ lightbulb (EXO TERRA, 2013). A cardboard box is self-crafted, where the lightbulb hangs from the top of the box, and each petri dish was put on the same

place under the lamp for an equal amount of radiation (Figure 1). The lightbulb irradiated the petri dish directly because UVB light can only kill the germs it contacts directly (Bryns, 2017 October).

Figure 1. UVB exposure box, self-crafted

Thirdly, each blood agar petri dish was divided into 3 equal parts, representing 3 trials. Bacteria colonies were counted, and the average number of colonies was calculated separately from these. The duration of UVB radiation, one of the independent variables, were 0, 1, 3, 5, 7 and 10 minutes, representing six settings. Exposing the petri dishes to the UVB lamp in different duration aimed to damage the bacteria culture in different scales. The number of minutes of UVB radiations were decided to intentionally harm the DNA of the bacteria, but not too long to kill them. Therefore, the highest number of minutes is 10 and is decreased in 2-minute intervals.

Fourthly, a glass rod, disinfected using ethane and gas heater, was used for transferring the 10-6 bacterial solution to the petri dishes instead of cotton swab. This was for preventing the possible contamination brought by other impurities in the cotton swab.

Finally, to answer the research question, the right conditions were provided for both direct reversal repair and nucleotide excision repair. Direct reversal repair requires certain wavelengths of light (350-500 nm) to activate the light-dependent process photoreactivation. Sunlight emits this range of wavelength needed in direct reversal repair and therefore six of the 12 petri dishes were placed under the sun for 4 hours after the UVB irritation. Nucleotide excision repair, however, does not require sunlight. Therefore, another six of the petri dishes were immediately covered in aluminum foil (Figure 2).

Figure 2. Sunlight exposure (foil covered the dark repair dishes)

The experimental method of this study consists of five phases. The initial bacterial culture was prepared by collecting samples from the faucet, sink and soap dispenser of school's public toilet. The reason for obtaining the microbial samples from a bathroom space, in addition to being relatively easy access to micro-organisms, was primarily for the purpose of replicating the potential microbiota found in a hospital. This has significance as the results of the experiment try to explore a microbe-safe option for surgical instrument preservation.

Then, a petri dish was covered with bacteria and was left to incubator to grow for 48 hours. After the 48 hours, yellowish *E. coli* bacteria colonies were observed in the petri dish, which was then later diluted using serial dilution, to get less concentrated bacteria source. To do this, bacteria colonies were collected with a cotton swab from the initial culture, which then is soaked in the tube containing 10ml deionized water for five minutes. The first test-tube contains full strength stock. 1ml of the first solution is taken from the first tube and added to the second tube containing 9ml of deionized water, which then contains 1:10 diluted bacteria. The above procedure was repeated until the result was $1{:}10^{-6}$ ratio of bacteria solution. Accurate micropipettes with

accuracy of 30-300 µl (± 4.0µl) and 10-100 µl (± 0.80 µl) were used in this stage.

The diluted sample of bacteria was then cultured into blood agar petri dishes. 12 blood agar petri dishes were marked by dividing each petri dish into three parts and with specific numbers that represent the number of minutes the petri dish was exposed to the UVB lamp. The petri dishes that will be exposed to sunlight after UVB radiation were marked LE and the ones do not were marked NO. About five centimeters from the tip of the glass rod was dipped into ethanol for a few seconds and then held to the gas heater. The ethanol on the tip of the glass rod burned into flame, and the flame died out eventually when the ethanol was completely combusted. The disinfected glass rod was dipped into the diluted bacteria solution and then gently swabbed over the petri dishes and turned 90 degrees to swab again. Each three parts of the divided petri dish was done part by part.

UVB exposure phase was followed immediately after the transfer (Kouassi, et al., 2017). The petri dish was put into the box which was closed immediately. Taken out after the specific minutes were passed. After the exposure, both the dishes marked LE and NO (covered immediately with aluminum foil) were moved next to the window, where there was direct sunlight available. After four hours of sunlight exposure phase, all the petri dishes were moved into the incubator. The incubator was kept at 27 Celsius degrees Celsius and petri dishes were left there to grow for 48 hours. After the 48 hours of incubation, all the petri dishes were taken out of the incubator and the number of yellowish colonies of bacteria was counted and recorded.

3. Results

Counting colonies could be performed manually using a pen and a click-counter. This is generally a 200 straightforward task but can become very time-consuming and is altered to high uncertainties when many plates must be counted. Alternatively, semi-automatic and automatic solutions can be used. Counting the colonies in this study was done manually using a pen. Each colony ‘’dot’’ was counted once. Below are the counted number of bacteria, average and standard deviation of each petri dish (Table 1).

Table 1 Firsthand raw data of the experiment, showing the number of bacteria colonies in all the individual petri dishes, in either sunlight exposure or darkness and in different amount of UVB-irritation durations (min).

Condition	0 min	1 min	3 min	5 min	7 min	10 min
Sunlight	138	141	147	132	252	94
Sunlight	160	103	209	122	184	91
Sunlight	141	112	171	129	139	115
Standard deviation	9.57	16.21	25.53	4.19	46.45	10.68
Dark	225	150	37	94	119	106
Dark	189	94	109	140	132	64
Dark	241	107	101	95	86	71
Standard deviation	21.75	23.93	32.22	21.45	19.36	18.37
Condition Average	0 min	1 min	3 min	5 min	7 min	10 min
Dark	218	117	82	110	112	80
Sunlight	147	119	176	128	192	100

As some overall trends, there are more bacteria colonies in the condition of sunlight exposure compared to the dark condition on each interval of UVB exposure (exception of 0-minute). The minutes of UVB-exposure seems not to have any significant impact on the number of bacteria being damaged and thus their repairing potential, especially in the sunlight condition (as an example, compare 3 min and 10 min from Figure 3). However, the regression line of the below graph helps for further analyses of significance of UVB exposure duration.

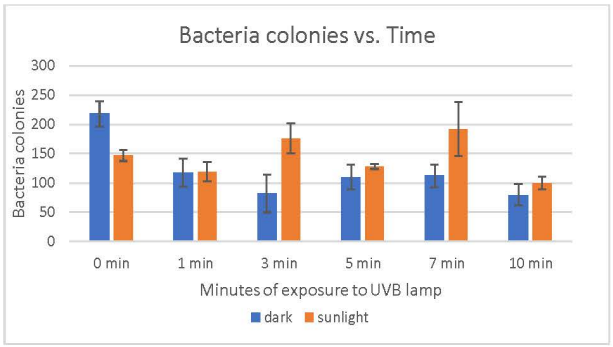

Figure 3 Comparison of average bacteria amount in dark and photo repair, error bars stating standard deviation

The regression line of sunlight condition is relatively even compared to the dark condition, where the regression line is decreasing significantly (Figure 4). The 6 conditions represent the independent variable, duration of UVB exposure. The R^2 values for the regression line for the sunlight repair is shown to be only 0.0095 while dark repair's 0.5107. R^2 represents the proportion for variation of dependent variables in respect to an independent variable, in a linear model (Hayes, 2020). R^2 value of both light and dark conditions suggest that time of exposure have had an effect on the repairing potentials, although the decrease in the dark repair is more than 50 times compared to that of sunlight exposure. This opposes the statement made previously, where the duration of UVB was thought to have no effect on the repairing potentials. The clear decrease of dark repair regression line might refer to the possibility of decreasing on the potential of nucleotide excision repairs the longer bacteria were exposed to UVB radiation.

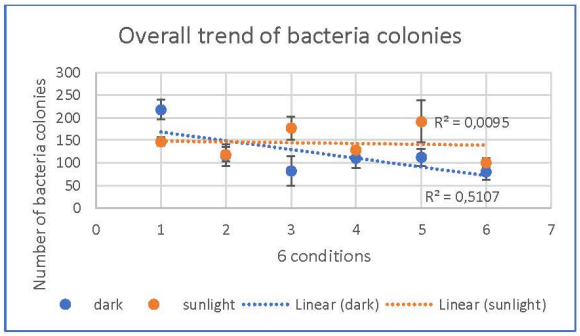

Figure 4 Overall trend of bacteria colonies in each condition, error bars stating standard deviations

Two-Way ANOVA was used in analyzing the data collected from this experiment because it is a useful tool when 230 the relationship between the quantitative variable (in this case the dependent variable, number of bacteria colonies) and two independent variables of the study needs to be compared and clarified (Fisher, 1921). Sample, Column and Interaction Within holds statistics that were applied to the results and raw data, which was then interpreted and analyzed. A significance level (denoted as α of alpha) of 0.05 indicates a 5% risk of concluding that a difference exists between the two independent variables when there is no actual difference (Minitab Express Support, 2019). The p-value from Table 2 is compared to the significance level of 0.05 (5%). This is to determine whether sunlight and dark setting (one of the two independent variables) have had a statistically significant impact on the average number of bacteria colonies. In other words, the ANOVA test helps to figure out whether the hypothesis should be accepted (Fisher, 1921).

The Interaction p-value, the most significant set of value from the ANOVA p-values (Table 2), determines whether the difference between effects of independent variables (sunlight vs dark) on the dependent variable (bacteria colonies), are due to a chance. The p-value of Interaction is significantly below α value as shown in Table 2 ($0.000631 << 0.05$). This is evidence of significant difference being existed between the effects of independent variables on the dependent variable which is not due to a chance.

The unprocessed data itself shows a certain pattern on the correlation between the number of bacteria colonies and exposure to sunlight or kept in the dark. Excluding the dish which was not exposed to UVB-lamp, all the rest sunlight exposed dishes show more bacteria colonies than the adjacent dark treated petri dishes. Besides this, the p-value being significantly lower than 0.05 supports the hypothesis of the study where it was stated that photo repair is more effective than dark repair. Given the same amount of time, direct reversal repair showed more repairing results compared to nucleotide excision repair.

An alternative suggestion for this result could be of energy-efficiency difference between two repairing mechanisms. As the name suggests, direct reversal repair directly reverses the damage in an error-free manner (Britt, 1995). In direct reversal, pyrimidine bases fused by UVB are separated by DNA photolyase. Photolyase enzymes in the photoreactivation contain light-harvesting cofactors, MTHF. It also contains FAD, an essential catalysis for the DNA repair (Liu, et al., 2015). Absorbing light (350-500nm) from sunlight excites the MTFH and therefore transfers electrons to FAD. Reduced FADH2 in turn, transfers the high energy electrons to dimers and breaks them by making the dimers highly unstable, and spontaneously decomposed to monomers (Kavakli et al., 2019). Direct reversal repair is specific to the damage and is the simplest form of DNA repair and the most energy-efficient

method.

Unlike photoreactivation, nucleotide excision repair does not directly reverse DNA damage but instead uses new, undamaged nucleotides to replace these damaged DNA. The damage is removed by cutting the sugar-phosphate backbone and from a certain site of the helix. At the site of the cut, a new segment displacing the DNA segment is synthesized by the DNA polymerase and joined by DNA ligase (Augusto-Pinto et al., 2003). This requires more energy and is comparably slower. This difference between energy requirement and repairing efficiency might have had led to NER having fewer repairing results than direct reversal repair.

Table 2 The results of Two-Way ANOVA test

ANOVA: Two-Factor							
SUMMARY	0 min	1 min	3 min	5 min	7 min	10 min	Total
Sunlight							
Count	3	3	3	3	3	3	18
Sum	440	356	527	383	575	300	2581
Average	146.666	118.666	175.666	127.666	191.666	100	143.388
Variance	137.333	394.333	977.333	26.3333	3236.333	171	1662.252
Dark							
Count	3	3	3	3	3	3	18
Sum	655	351	247	329	337	241	2160
Average	218.333	117	82.333	109.666	112.333	80.333	120
Variance	709.333	859	1557.33	690.333	562.333	506.333	2839.882
Total							
Count	6	6	6	6	6	6	
Sum	1095	707	774	712	912	541	
Average	182.5	117.833	129	118.666	152	90.166	
Variance	1879.5	502.166	3627.2	383.866	3407.666	386.966	
ANOVA							
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Sample	4923.36	1	4923.36	6.01183	0.021866	4.25967	
Columns	30523.1	5	6104.62	7.45426	0.000242	2.62065	
Interactions	26358.4	5	5271.69	6.43718	0.000631	2.62065	
Within	19654.6	24	818.944				
Total	81459.6	35					

An unexpected outcome was also detected in this experiment. While all the other petri dishes with a certain amount of UVB radiation showed more bacterial growth with sunlight radiation than the ones left in dark, the petri dishes with 0 minutes of UVB radiation showed a contrariwise result (refer to Figure 3, the condition with 0 min UVB exposure). It is observed to have more bacterial growth while left in the dark, and less left under the sunlight, which is against the general trend with other petri dishes. Educated guesses and alternative explanations can be driven for the reason for this observation. UVA has the longest wavelength of 320-400 nm with lower energy and is responsible for 95% of the UV radiation that reaches the Earth's surface. Although UVA is less intense and harmful, it is 30-50 times more prevalent (Chien & Jacobe, 2019). Thus, it could be reasoned that when the bacteria culture is left under the direct exposure of sunlight for four hours, the UVA radiation coming from the sunlight have had damaging effect rather than repairing effect on the DNA of the bacteria. As mentioned in the

article of Girard (2011, p.261), *UVA-induced damage to DNA and proteins: direct versus indirect photochemical processes:*

"Unlike UVB, the UVA component of solar radiation is weakly absorbed by DNA, but rather excites other endogenous chromophores, generating various reactive oxygen species (ROS) in cells… Once thought to be relatively innocuous, UVA is now known as a damaging agent for DNA, proteins, lipids, with harmful consequences such as skin aging and carcinogenesis." (Girard, 2011)

4. Conclusion

The results of this study successfully answer to the research question by comparing direct reversal repair and nucleotide excision repair and their effectiveness on repairing UV-induced damage. As predicted in the hypothesis, the results of this experiment indicate that direct reversal repair where visible light from the violet/blue end of the spectrum activates photolyase is relatively more effective than nucleotide excision repair in the dark.

For backing up the research and the data sufficiently, multiple relevant scientific articles from peer-reviewed journals, research reports and books have been reviewed and used as sources. Especially these two articles amongst many others have provided this study with valuable insights: *Repair of DNA Damage Induced by Ultraviolet Radiation* (Britt 1995) and *The Mechanisms of UV Mutagenesis* (Ikehata & Ono, 2011). It is kept in mind and ensured that the sources used need to be valid and reliable, and critical thinking and reading were demanded while reviewing and using these articles.

The findings of this research could be applied on further improving the UV radiation water treating technology and medicine surgical instruments, to beware of the possible revival of dangerous pathogens. The research indicates the repairing under sunlight is more of a concern, and that possible sunlight exposure after the UV treatment should be avoided. Further research could be performed to study if temperature changes affect the repairing potential of the repairing mechanisms. This would provide a viewpoint to other aspects of the surrounding environment, in addition to light. There are previous studies done on similar variables, making it possible to compare the results to the previous papers (Salcedo, et al., 2007). Further studies could be done by introducing other exogenous DNA damages, such as environmental and chemical carcinogens, and compare the repairing effectiveness of DNA repairing mechanisms. Also, if the possibility is provided, measuring the CFU of bacteria sample in each time interval during the incubation phase would allow to produce a line graph where it is possible to determine the rate of each repair mechanism.

5. Evaluation

The first experiment failed due to too high concentration of bacteria. The bacteria taken from the first agar culture was diluted to a ratio of 1:100, which was too concentrated, and the different independent variables were not able to have an impact on it. The experiment was therefore done again. Before directly culturing again and waste too much petri dishes, it was decided to test the concentrations first on a petri dish divided into three parts and each part growing bacteria with different concentrations. Then, the procedures that were done previously were repeated, but with the 10-6 solution.

The bacteria grown in the petri dish was assumed to be *E. coli* because the blood agar petri dish is an ideal environment for *E. coli* to grow. However, all the colonies are not necessarily *E. coli*, there might be other species involved that is not known but still counted into the result of the study. This could be solved by purchasing ready *E. coli* solutions, as it was done with blood agar petri dishes.

As mentioned in the introduction, two main types of repairing mechanism might have taken place when the petri dish is covered in aluminum foil and left in the dark. Even if defining NER and BER to be different on repairing internal or external damage separately, the study and its setting cannot be sure on which of the repair has taken place or if both have occurred. This effects the discussion and data analyses significantly. Alternatively, different environmental and other chemical mutagens could be introduced to damage the bacteria DNA to make sure that the risk factor is strongly exogenous, for the requirement of NER.

Counting the bacteria colony manually introduced

uncertainties to the research data. One bacteria colony might have been counted twice due to many colonies and distractions. Automated counting using certain software might give more precise numbers but has its risks. For example, algorithms can have difficulties differentiating colonies when two or more colonies are touching at the edges or their results are easily affected by uneven illumination and reflection of visible light (Zhu, et al., 2018). Better option to make sure the accuracy of the data could be counting the colonies both manually and with software, and possibly take their average if not too high standard deviation occurs.

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Analysis of SARS-CoV-2 Sequences Reveals Transmission Path and Emergence of $S^{D\ 614G}$ Mutation

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Abstract

The coronavirus disease 2019 (COVID-19) outbreak caused by SARS-CoV-2 virus began in Wuhan, China and spread quickly throughout the world. The development of vaccines for SARS-CoV-2 is difficult due to many obstacles, such as the lack of knowledge of important proteins, genes, and mutations of the viral genome. In this study, we selected and utilized 852 strains of COVID-19 from major countries in the National Center for Biotechnology Information (NCBI) global virus bank. The information of these strains was processed by using Nextstrain software, a program that provided a visual phylogenetic tree, transmission map, and diversity panel that explains entropy and number of mutations for each codon in the genome. The general data about the spread and evolution of COVID-19 supported the current knowledge that it began in China and spread throughout the country in an interrelated manner instead of a clear "patient zero" manner. A recent study reported that codon 614 on COVID-19 spike protein (S614) was an important codon for viral spread, specifically, a mutation from aspartic acid to glycine facilitated the spread of the virus. Therefore, we chose to geographically track this mutation during the spread of COVID-19 to investigate where it emerged and whether it can affect the spread COVID-19. Our results showed that the glycine mutation first emerged in France and that the transmission rates in France versus China (where the mutation was not prevalent) did reflect the hypothesized change in viral behavior.

Keywords: SARS-CoV-2, COVID-19 Spike Protein, Nextstrain

1. Introduction

In December 2019, viral pneumonia cases were reported in Wuhan, China, eventually identified as novel coronavirus cases known as coronavirus disease 2019 (COVID-19). This infectious RNA virus, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), began spreading globally in January 2020. On March 11, 2020, the World Health Organization (WHO) declared COVID-19 a pandemic (Cucinotta & Vanelli, 2020). Therefore, COVID-19 is an urgent global conflict. As of early July 2020, there were over 13.2 million confirmed cases and 575,000 deaths in 216 countries due to this virus (World Health Organization, 2020). COVID-19 likely emerged from bat-associated coronaviruses around November 2019 that can be mutated in a host (Zhou et al., 2020). The high mutation rate leads to difficulties in developing vaccines (Pathan et al.,

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2020). Therefore, this study is aimed to investigate COVID-19's evolution and mutations through a timed phylogenetic tree built in Nextstrain, an open-source program that processes and visualizes public pathogen genome data for scientific and public health implication.

We aimed to identify and analyze the evolutionary relationship of COVID-19 strains around the world by using Nextstrain program. The program encompasses phylogeny, transmissions, and diversity features that represent the geographical and biological interactions among various strains. The system illustrates the changes and constants of the strains from December 2019 to June 2020 publicly available on the National Center for Biotechnology Information (NCBI) Virus database. From a single dot in Wuhan, China, in late September 2019, transmission lines began to grow by late December 2019. The visuals through colorization in various countries establish a multi-colored transmission web throughout the world. The interactive graphics supported by Nextstrain that potentially aids in COVID-19 knowledge includes a phylogenetic tree, a transmission map, and a diversity index.

By identifying and analyzing COVID-19 evolutionary relationships will improve our understanding of how the virus mutates and transmits and help the development of vaccines and methods of combatting the pandemic. Numerous clinical trials began, and the United States government has established Operation WARP Speed all in effort to speed up the vaccine process. The evolutionary relationships will aid in the safety and effectiveness of vaccines in the shortened procedure (Harvard Medical School, 2020). There are obstacles for developing the vaccine, as there are many ambiguities about the future of COVID-19 since the virus is new and the present understanding rapidly changes. Creating interim guidelines for COVID-19 antibody tests, the Centers for Disease Control and Prevention (CDC) has advised healthcare providers, laboratories, and public health staff when looking for antibodies, proteins produced after infection in the blood (Centers for Disease Control and Prevention, 2020a). Although a positive antibody test generally indicates a SARS-CoV-2 infection, the CDC cautions that there is not enough information to assume that an individual is immune from the virus with the antibodies. As of early July 2020, there have been many treatment and prevention attempts, including dexamethasone, vitamin D, chloroquine, hydroxychloroquine, and azithromycin. Additionally, antiviral drug remdesivir has been used in adults and children with possible and confirmed COVID-19 infection (Yale Medicine, 2020). However, the effects of these treatments are not satisfactory. Therefore, this study aimed to bridge the gap between the current knowledge and future implications by visualizing public global data from NCBI and analyzing current viral mutations between December 2019 and June 2020.

Our goal was to use Nextstrain services and our selected data to confirm current knowledge on COVID-19 transmissions. We investigated whether or not the theory that there is no "patient zero" was true. We aimed to gain a better understanding of the biology of COVID-19 and focused on specific codon mutations that have been tagged as important proteins to COVID-19 viral behavior. Because one of Nextstrain's keynote features is its transmission map, we tracked the mutation emergence and path.

2. Methods

Due to the political nature of COVID-19 test, it was important to ensure that the strains collected were accurate and credible. We defined this standard of strains as being from the NCBI database, having a complete or nearly complete sequence of over 29,000 bases, and being published by the NCBI consortium (NCBI Virus, 2020). Based on the standards, we selected and formatted 580 sequences from the countries in the NCBI database with the most strains available. The majority of the sequences selected were from 2020, as the majority of countries affected by COVID-19 were affected in 2020, but approximately 7 strains from China were from 2019. All of the strains were SARS-CoV-2 strains from human hosts. We then added 580 sequences to the existing Nextstrain global database of human strains for a total of 997 sequences.

While running Nextstrain, we filtered and aligned the sequences. The filters removed any improperly formatted sequences as well as sequences with excessive absent bases. The remaining 852 strains were selected to create phylogenetic tree.

After aligning the sequences, Nextstrain returned a list of base mutations, and these mutations are the basis of the phylogenetic tree (Figure 1). The remainder of the commands used to create the phylogenetic tree revolved around using Auspice and Augur, two subprograms of Nextstrain, to visually create the phylogenetic tree. Once this tree was exported, the visual phylogenetic tree, transmission map, and diversity index, were available (Figure 2).

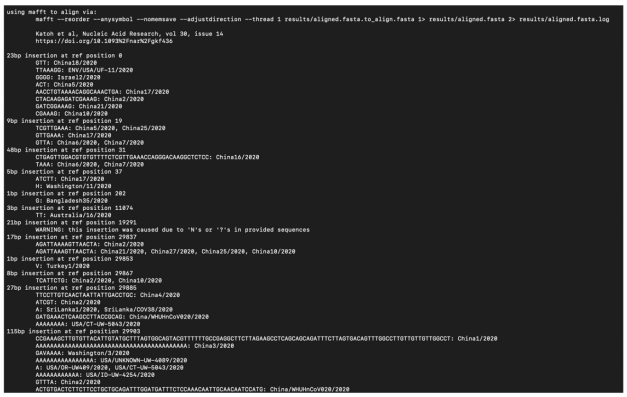

Figure 1. *Processing the SARS-CoV-2 data with Nextstrain.* This figure shows a list of mutations, mostly insertions, that occurred in the strains used as input into Nextstrain. This computer-generated list is the basis for the phylogenetic tree. It is also another filtration step as the program filters out strains with large amounts of unknown regions.

3. Results

After inputting and processing the 852 SARS-CoV-2 strains, Nextstrain presents three panels with the phylogeny, transmissions, and diversity that visualizes the viral relationships. The Nextstrain features, supported by Auspice and Augur, are demonstrated in Figure 3.

The phylogeny feature represents the evolutionary relationships among different SARS-CoV-2 sequences on the molecular level by the observed mutation patterns. The x-axis depicts the degree of difference in time or genetic divergence, while the y-axis spreads the sequences for better visualization. To measure the differences over time, x-axis reflects each sampling date, with the tree's tips also representing the sampling date. Nextstrain infers the internal node dates, the "missing cases" that are intermediate and unsampled, through their descendants' sampling dates and viral mutation rates. Even though the sequences may have similar mutations, the tree by date accounts for both the mutation and spread rates. The genetic divergence differences compare the number of mutations to the estimated start of the outbreak and organize the number of changes in the genome. The identical sequences are grouped together on the tree.

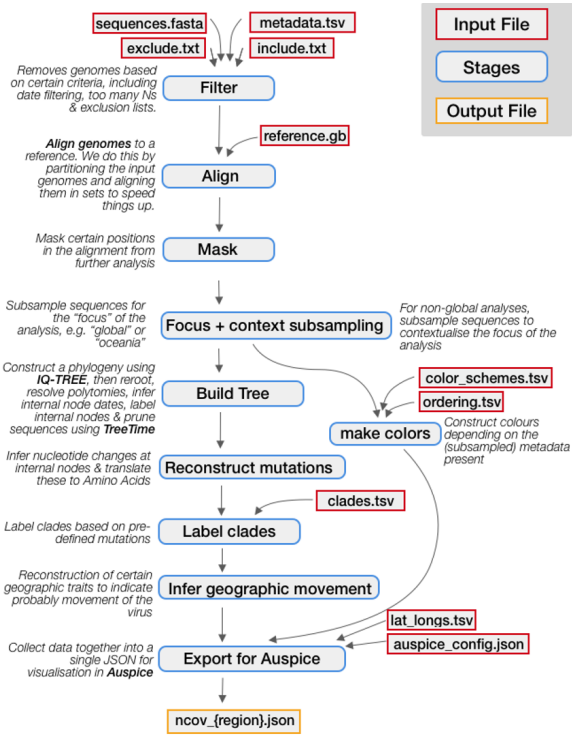

Figure 2. *Steps to Process the SARS-CoV-2 data with Nextstrain.* This figure shows the steps in inputting, processing, and outputting the data with the Nextstrain program.

The transmission networks represent how the pathogens, SARS-CoV-2 in this instance, spread through rapid replication from one host to another. The genome sequences help intersections of the transmission tree. Through replication and spread, the pathogenic genome experiences innumerable replication cycles, inevitably causing mutations, which are random copying mistakes. The sequences with similar mutations are more closely related than others, which allows the program to organize them into groups of associated viruses that are a part of the same transmission chains. Nextstrain colors the phylogenetic tree by the sample location and suggests

viral spread throughout the outbreak. Therefore, these genome alterations accumulate, help track the spread, and establish the pathogen's routes and interactions.

As demonstrated by the "diversity" panel, the program uses the variations in the genome, including mutations in nucleotides and amino acids, to construct the phylogenetic tree. The bar-chart has a horizontal axis that includes all the viral genome sites, which is approximately thirty thousand, and a vertical axis that reveals how much variability is at each site. Although there is no reason to believe that each alteration is a functional mutation, Nextstrain encompasses a feature that colors the tree by a mutation since the program uses the changes to organize and define the relationships of the sequences to build the tree.

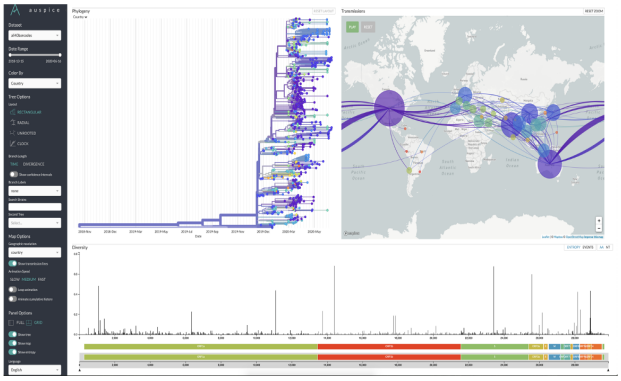

Figure 3. *Nextstrain panels after processing 852 SARS-CoV-2 strains.* This figure shows the panels of Nextstrain after the data of 852 strains was inputted and processed. In the first row, the phylogenetic tree and transmission map are pictured. In the second row, the diversity panel is pictured with "entropy" selected, so it is a measure of uncertainty of each of the codons on the genome. A visual representation of the genome is pictured below the diversity index as its x-axis.

The Nextstrain mapping reflects the common knowledge about the spread of COVID-19, in that it began its path from China with its inferred starting date in October 2019. When the date is unknown, Nextstrain assigns the outbreak start date by using the available dates from each sample and node in the phylogenetic tree, pictured in Figure 4. This "root" of the tree represents the "most recent common ancestor" for all of the SARS-CoV-2 sequences available thus far. The transmission path of the virus is pictured in Figure 5a. With the virus beginning in China with 100% confidence, the first transmissions occurred in Germany and Australia, followed by Thailand, South Korea, Taiwan, Israel, and Nepal in December 2019. The first transmission to India was in January 2020. By mid-January 2020, the virus travelled to France, Egypt, Hong Kong and Japan. Towards the end of the month, the United States, Italy, Pakistan, Chile, Kazakhstan, and Guam had SARS-CoV-2 cases. In February 2020, Spain, Peru, Greece, and Saudi Arabia had transmissions of the virus. In early March 2020, Brazil, Turkey, and Sri Lanka faced the virus. By mid-March 2020, Jamaica, Iran, Malaysia had COVID-19 cases. By the end of the month, there were SARS-CoV-2 strands in Uruguay, Bangladesh, and Timor-Leste. Between April and June, there were no particular transmissions, but the viral cases grew and diminished throughout the months, including transfers from countries other than China.

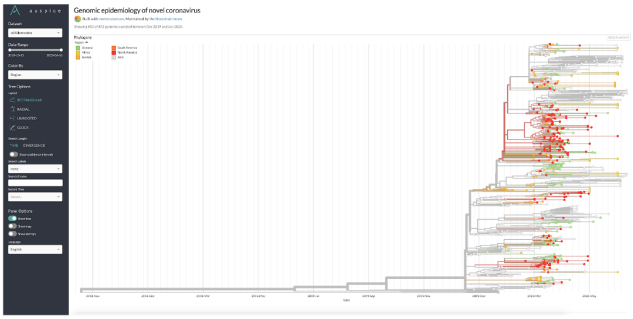

Figure 4. *Nextstrain phylogenetic tree with 852 SARS-CoV-2 strains.* This figure shows a panel of Nextstrain after our data of 852 strains was inputted and processed. The phylogenetic tree highlights the regions through color-coding to understand the origins of each strain, ranging from October 2018 to June 2020.

From the diversity panel in Figure 5b, we found that a significant majority of codons had only one mutation. Furthermore, there were only 3 codons with over 4 mutations. Although further studies are necessary to understand whether these codons are significant to COVID-19's function, the overarching summary is that COVID-19 is relatively stable and doesn't have a large number of mutations. Among the mutations, the majority are not prevalent and

likely occur due to replication errors or sequencing errors.

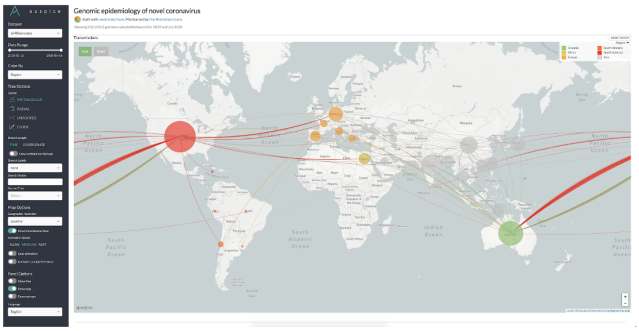

Figure 5a. *Unique Transmission Map*. This is the transmission map of the data color coded by region. The size of the circles reflects the number of sequences used from each country, and the lines from country to country signal how the strains spread.

Figure 5b. (below) *Diversity Panel with Events*. This is the diversity panel of the data we entered into Nextstrain. However, in this panel, "events" is highlighted, which means that the bars are noting the number of mutations that occurred on each codon of the genome. Again, the genome is pictured as the x-axis of the panel.

4. Discussion

Significant Findings

We used the mapping feature to track genes that have a significant impact on COVID-19's structure and function. For example, researchers of the Scripps Research Institute documented that the S614 gene on the COVID-19 spike protein had a mutation of aspartic acid (D) to glycine (G) (Zhang et al., 2020). The glycine made COVID-19 more stable, and thus made it spread faster. We tracked the S614 gene spread to visualize where the mutation began. We found that the glycine mutation began in France in early January 2020 (Figure 6a). Our Nextstrain build estimated that the mutation emerged with the date confidence level between January 5 to January 23, 2020 (Figure 6b) and later spread through Europe, then to the rest of the world (Figure 6c). According to our Nextstrain diversity index, the mutation is on codon 614 located on nucleotide positions 23402 and 23404 in protein S due to an entropy spike of 0.677 (Figure 6d). This is corroborated by much higher spreading rate in France than in China's first exponential growth (New York Times, 2020). This is significant because it shows that the mutation was not present in China during the virus's initial spread and that it presented itself in the viral behavior, or its spread.

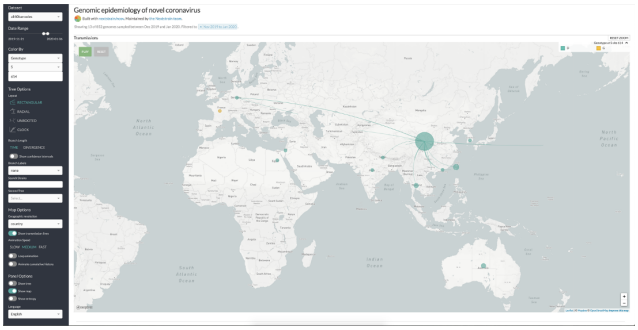

Figure 6a. *Beginning of S614 mutation transmissions map*. The figure represents that France is the first country to experience the S614 mutation in early June, color coding the strains with the mutation as teal and without the mutation as yellow.

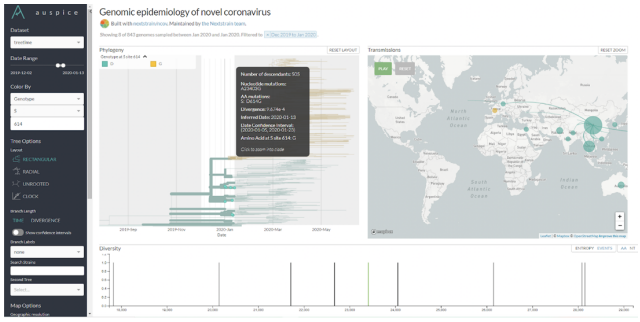

Figure 6b. *S614 mutation phylogeny.* The figure color codes the strains with the mutation as teal and without the mutation as yellow.

Methods Used

Nextstrain, the algorithm we used, worked well, in that it provided us insight into both the general COVID-19 transmission pathway and the specific S614 gene mutation. This is likely due to the

geographic element of Nextstrain that separates it from other phylogenetic tree compilers— it allowed us to track the gene mutations and transmission in a way that would be difficult without the clear geographic map. As the virus spreads, the program tracks small changes in the viral genome that aids in understanding the global movements.

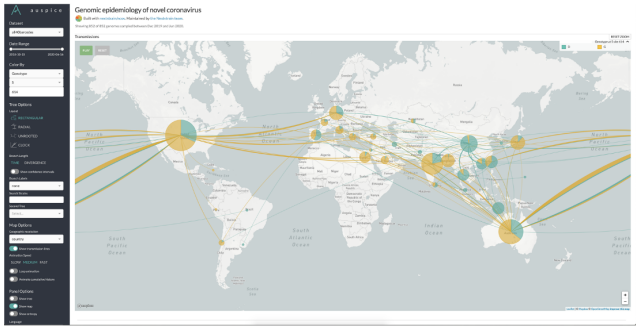

Figure 6c. *S614 transmissions map.* This figure is a world map representing the S614 mutation transmissions and divergence through its whole pathway. There is color-coding of the strains with the mutation in yellow and those without in teal.

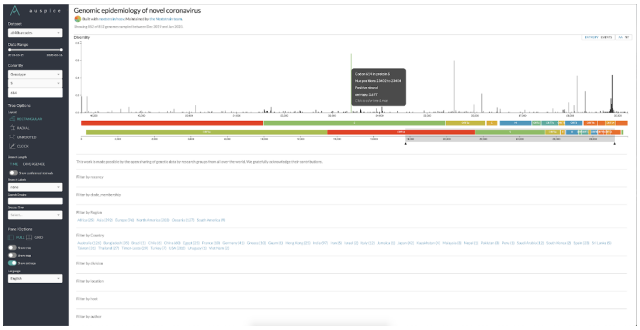

Figure 6d. *Diversity data focusing on entropy and amino acid sequences.* This figure demonstrates that there is a mutation on codon 614 located on nucleotide positions 23402 and 23404 in protein S due to an entropy spike of 0.677.

Rationale for Results

SARS-CoV-2 virus has RNA as its genetic material that contains nucleotides in codons as its building blocks. Upon infecting a cell and making copies of its genetic instructions, viruses are constantly changing and generally do not have the required machinery to proofread their replicated RNA string for errors, resulting in various genetic accumulations (Garcia de Jesus, 2020). Unlike most RNA viruses, the *Nidovirales* order, which the Coronavirus genus belongs to, has the proof-reading capability, allowing them to have the largest RNA genomes. The order has a complex machinery for RNA synthesis that is operated by nonstructural proteins (nsps) to produce cleavage products of the ORF1a and ORF1b viral polyprotein to coordinate virus replication and transcription. With a high homology for SARS-CoV-2 RNA-dependent RNA polymerases (RdRps) used in replication and transcription, SARS-CoV-2 has conserved the machinery, demonstrating its importance (Pachetti et al., 2020). Despite these tools to prevent mutations, these changes can accumulate that may be slower than other RNA viruses without the enzymes such as influenza that results in mutations including S614 mutation. As stated before, the mutations are useful in tracing the virus throughout the world. For SARS-CoV-2 cases, researchers have been analyzing the viral path since the release of the first coronavirus genetic sequence in January 2020, allowing them to sequence the RNA changes as it spreads and infects more people even if they do not alter the protein (Garcia de Jesus, 2020).

Represented by the transmissions map, SARS-CoV-2 has been spreading throughout the world since the end of December of 2019. However, the understanding of how the virus spreads in mid-July 2020 is through person-to-person contact and respiratory droplets within about 6 feet (Centers for Disease Control and Prevention, 2020). The Nextstrain highlights the hypothesis by presenting more global transmissions before mid-March 2020 when WHO declared the virus as a pandemic.

Comparison to Other Results

Compared to Nextstrain novel coronavirus (ncov) built with 3,409 strains, our initial results regarding the spread of COVID-19 were very similar to the transmission map for Nextstrain ncov. For the S614 mutation, however, there was a slight difference. We found that France was the first location for glycine in S614, while the ncov build showed Italy and Finland as the primary location. This is likely due to smaller sample size of strains in our study, but in both situations the emergence was in Europe, which had a higher spread rate than in China (Figure 7).

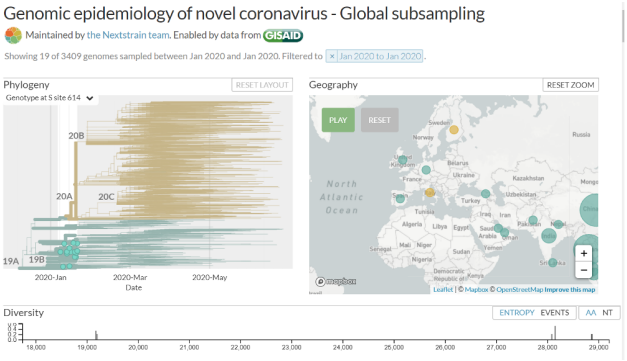

Figure 7. *ncov phylogenetic tree and transmission map*. The above image is the phylogenetic tree and transmission map panels of the data produced by the Nextstrain ncov build. They show that the glycine mutation emerged in Italy and Finland in mid-late January.

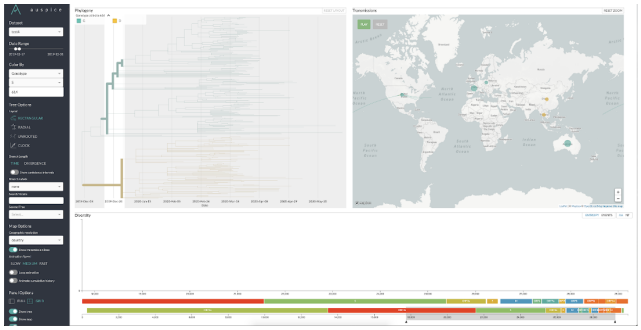

Figure 8. *Test build phylogenetic tree and transmission map*. The above image is a paused frame of the test build phylogenetic tree and transmission map. It shows that the glycine mutation began in China or Thailand, although this is not nearly as accurate as the ncov or our data samples as it is a much smaller sample size.

Additionally, although there are no other publications about S614 transmissions, the information about the COVID-19 spread in France and China did correlate with the aspartic acid to glycine mutation. Specifically, it makes sense that France, with the glycine mutation arising, would have a higher rate of cases compared to China due to the nature of the S614 mutation.

Limitations

First, the main limitation is the data collection. Although some countries have a large number of cases, they do not necessarily have a large number of strains in the NCBI database. For example, the United Kingdom, Brazil, and Russia are three countries that have a large number of cases but have very few strains: 0, 5, and 9 strains in the database, respectively. This occurrence does leave these countries out of analyses and interpretations, especially if they do not have any strains in the database. Although there was an attempt to select strains from nearby or within all countries with a significant number of COVID cases, the NCBI database limited the possibility.

Second, in the countries with many strains available for download there were strains from the same date with consecutive accession numbers, potentially signifying that the data does not have significant differences and cannot demonstrate the evolution of the virus within the country. The date ranged from December 2019 to June 2020. To combat this, during sequence selection, we did try to ensure that the strains selected were not all from the same date. However, in the case where the only sequences from a country were from one date, we kept that in consideration when looking at our phylogenetic tree and analysis.

Third, the spreading information we used is limited by the data provided by the countries, which is not necessarily accurate due to either lack of testing or lack of reporting accurate data.

5. Conclusion

Using the phylogenetic tree and transmission map created by the Nextstrain algorithm help understanding the overall spread of Covid-19. We found that D614G mutation on the COVID-19 spike protein emerged in France in early January and spread throughout the world. This is correlated to increased rate of spreading in Europe compared to China in the early stage of infection.

Future Work

Mutation emergence should be further investigated, especially in conjunction with epidemiology and microbiology. For example, once more codons and proteins are understood, they can be tracked to understand virus evolution. If these codon mutations are important to vaccine development, using Nextstrain builds can show how they affect the countries where the mutation was prevalent.

Additionally, regarding D614G mutation, our methodology should be reproduced in the future with a different, larger set of data specifically from Europe and Asia to have a better understanding of where this mutation started.

Acknowledgment

This project was supported by Indianapolis Project STEM and sponsored by Indiana CTSI Project STEM for high school students. Thank you, Mr. Elmer Sanders, Dr. Huanmei Wu, Mr. Dwight Hall, Wu Lab group, and the Project STEM community for all your support during the Summer 2020 session.

Disclosure

ML and NP are high school students from Canterbury High School and Carmel High School in Indiana. They attended Indianapolis Project STEM 2020, a summer internship, under the mentorship of HW. They mainly focused on the biology section of bioinformatics, processing SARS-CoV-2 strains provided by the NCBI Virus consortium and using Nextstrain under the guidance of DW. ML and NP composed this manuscript to present their findings throughout their summer internship and hopes to expand the knowledge about the ongoing COVID-19 pandemic.

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Using Simple Behavioral Analysis to Create Novel Universal Social Defeat Chronic Stress Classifiers in Mice Behavior

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Abstract

Anxiety and other mood disorders are extremely prevalent conditions affecting over 70% of high school students throughout the U.S. Chronic social defeat stress is a debilitating type of stress that causes significant changes in behavior and brain functioning. Developing animal models to study these disorders can revolutionize how they are treated by providing more about the biological bases of anxiety. Currently, developing these useful animal models for psychiatric disorders is a major challenge due to bias from the experimenter. The hypothesis of this project is to see if a successful universal classifier for chronic defeat social stress can be developed. Using a library of videos to observe the self-grooming and rearing behavior of mice (behaviors associated with depression and anxiety), the frames of the behavior were recorded each time the respective behaviors were exhibited. This data was fed into simBA (Simple Behavioral Analysis) which is a deep neural network that recognizes body parts of animals in each frame. Body distance and movement data were obtained, and the mean, standard deviation, and r^2 values were calculated. The results indicate that two successful and novel universal classifiers were created. Thus, universal classifiers should be considered as a viable method for examining depression and anxiety behaviors in mice.

Keywords: Computational Neuroscience, Machine learning, chronic stress, mice models

1. Introduction

Research has shown that 1 in every 3 high school students in the U.S. diagnosed with mood disorders such as anxiety and depression (McCarthy, 2019), developing animal models to study these disorders could revolutionize the efficacy of potential treatments. A common observed source of this stress is chronic social defeat stress. Chronic social defeat stress is a source of chronic defeat in animals and humans. It is a concept that is used to understand the physiological and behavioral effects of hostile interactions between animals and humans. In humans, chronic social defeat stress is exhibited by behaviors such as depression, anxiety, low self-esteem, and other psychosomatic diseases. According to research, (Golden et al., 2011) social defeat chronic stress has been observed to cause significant changes to brain functioning and its physiology such as hormone and neurotransmitter levels. Specific brain structures such as the nucleus accumbens, hippocampus, and prefrontal cortex are majorly involved with stress and are therefore the structures of interest at the physiological level.

Additionally, mice models have the discriminative ability to distinguish animals on anxiety and depression like behavioral domains. In rodent models, social defeat is experimentally initiated when a male rodent is introduced into the home cage of an older, aggressive, dominant male. Repeated

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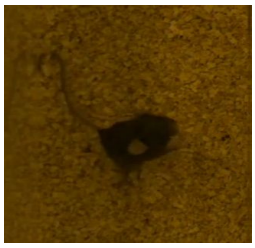
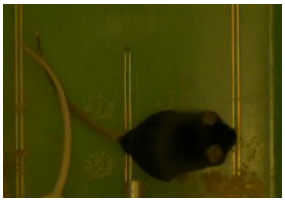
exposures to social defeat stress in rodents elicit anxiety and depression like behaviors which has comparative parallels to clinical behaviors found in humans (Golden, et. al., 2011). Neuroethology is the specific science of measuring these natural behaviors in order to further understand the brain. Currently, there is disparity in developing useful animal models for psychiatric disorders and to investigate the ethology is a major challenge due to subjectivity. In a research paper published by Nature Neuroscience, it was stated that "modeling of human neuropsychiatric disorders in animals is extremely challenging given the subjective nature of many criteria (Nestler & Hyman, 2010, vol.13). However, in a later study done by (Datta et al., 2019) it was found that the recent development of deep-learning based frameworks for tracking, is revealing the behavioral richness and variability that underlies even simple behavioral reports such as pellet grabs or lever presses. This evidence displays the importance of creating a novel universal classifier that eliminates subjectivity and is very prevalent because it shows how animal models will help potentially create more effective treatments for increasingly common mood disorders such as anxiety and depression. Therefore, the purpose of this research is to use a deep neural network machine learning program called Simple Behavioral Analysis (simBA) to create novel universal classifiers to identify particular depression and anxiety social defeat target behaviors in mice.

2. Methods

Using a computer with simBA, a library of videos of Mus musculus mice with chronic social defeat stress, exhibiting symptoms specifically relating to anxiety and depression were analyzed. These videos are provided by the Golden Neuro Lab. These videos show that a mouse acquires social defeat chronic stress behaviors when an intruder mouse is introduced in their cage. Of particular interest, the target behaviors in this project were self-grooming and rearing and were observed through the Mus musculus mouse behavior. Self-grooming is characterized by a maintenance behavior that consists of an uninterrupted grooming session where the body is curled, and the front paws are used to clean the head and body. Rearing is characterized as an exploratory behavior where the mouse stands on its hind legs alone or against a wall, looking up (See Table 1 for illustrations and depictions of self-grooming and rearing behaviors).

Table 1. This table describes how the behavior of the mice was analyzed for the corresponding classifiers.

Classifier	Description	Start Frame	Duration	End Frame
Self-Grooming Behavior 	Maintenance behavior that functions to maintain the physiological balance, comfort and appearance of the mouse.	Mouse curls body and begins sequence of licking and scratching with front paws.	Uninterrupted session of curled body and using front paws to clean head, face, and behind the ears or other areas of the body.	Intruder mouse resumes activity prior to grooming and/or goes back on all four paws.
Rearing Behavior 	Often represents inspective and cautious exploration of an environment	When the intruder mouse is standing on its hind legs either alone or against a wall and looking up.	Intruder mouse will continue standing on hind legs and looking upwards.	First frame that intruder mouse goes down on all four legs.

These behaviors were chosen because self-grooming is a comfort behavior that is seldom exhibited during times of stress while rearing is a bolder behavior that is more apparent in dominant mice compared to the stressed mice. When the intruder mouse was introduced in the cage, rearing was more common behavior. However, when there was no stimulus of the intruder mouse, self-grooming was more commonly observed, as that is a relaxed behavior. Each video is exactly 10 minutes and 18,000 frames long. The videos were observed using Windows Media software. Each instance of grooming or rearing behavior is observed during the video. Start and end frames are recorded for coding and scoring purposes for later analysis. Once all the videos are scored for the corresponding behavior, the data is fed into a program called Deep Lab Cut (DLC). To create the tracking model, use the behavioral annotator function in simBA (figure 1). This integrates a video player and a frame viewer and syncs them so that the frame viewer can be used to annotate when behaviors of interest are present or absent within the given frame. The observed behavior was coded for the classifier if the frame displayed the self-grooming or rearing behavior. The behavioral data for that particular frame is then saved.

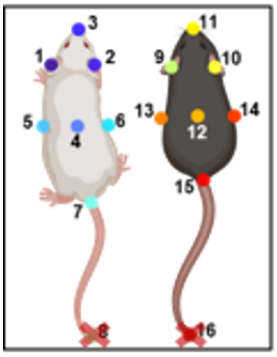


Figure 1. This figure displays how the colored dot parameters appear in simBA software in order to calculate the behavior.

Data Analysis

I first analyzed 30 videos with 18,000 frames each resulting in 540,000 total data points. I created the classifier by looking at each video being analyzed and tracking the observed mouse. This information is fed into the deep lab cut (DLC) program which generates a huge amount of data. Applying inter rater reliability, it was confirmed that the frames were reliable for predicting behavior and building decision trees. Once classifiers are created, descriptive statistics (mean, standard deviation, and R^2 values) could be obtained for behavioral predictions. I obtained descriptive statistics (mean, standard deviation, and R^2 values) for each classifier. Data were generated in an Excel spreadsheet which were then used to create a learning curve and a precision and recall graph. Precision attempts to address what proportion of the data was correctly classified as the behavior of interest. Recall attempts to address what portion of the classified behavior truly was correct. The R^2 test is important because it indicates that the model explains all the variability of the response data around the mean.

3. Results and Discussion

The data obtained was very strong as the precision and recall graph shown in Figure 2 displays that the R^2 value was 1.0 for 97% of the self-grooming classifier and 1.0 for 85% of the rearing classifier.

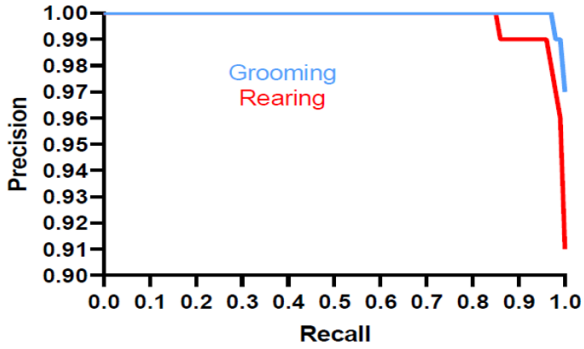


Figure 2. Precision and Recall Graph for rearing and grooming classifiers.

For the learning curves of both behaviors shown in Figure 3, the graphs for both classifiers were also strong because the graph showed that as the percentage of the video analyzed increased, the harmonic mean of the data rapidly increased and then started to level off. The standard deviation was also taken into account and displayed on the graph displaying the learning curve. The standard deviation for the self-grooming classifier didn't exceed 0.001 on average and for the rearing classifier didn't exceed 0.006 on average. The successful creation of two novel classifiers based on mice behavior is important

to future research because it shows the possibility of using technology as an intervention for identifying behaviors of interest.

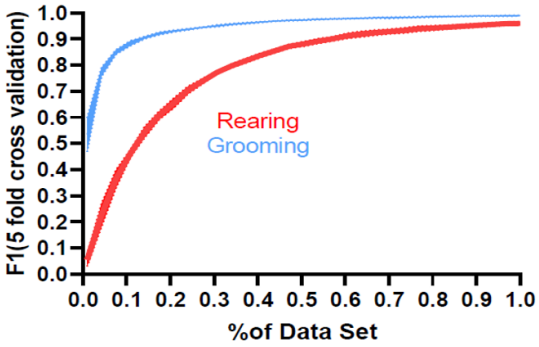


Figure 3. Learning Curves for rearing and grooming classifiers.

This can then be used to develop effective approaches to treating mood disorders relating to anxiety and depression. Research by (Golden et al., 2011) indicates that humans display chronic social defeat stress is in work and school places. It is exhibited by behaviors such as depression, anxiety, low self-esteem, and other psychosomatic diseases. With successful recognition of the stressors that cause these types of behavior and accurately identifying the behaviors of interest, therapies can be applied to minimize neural disorders such as anxiety and depression.

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Exploring the Visionary Mind: Review of Psychometric and Neuroimaging Tests to Examine Human Creativity

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Abstract

Creativity has long been an intriguing topic to philosophers, psychologists, educators, business executives, and, most recently, neuroscientists. Decades ago, studying human creativity had been challenging mainly because of a lack of suitable testing approaches to obtain subjective measures that could standardize and quantify an individual's creative thinking abilities. Recent advances in research techniques have allowed researchers and scientists to scientifically evaluate creativity in various domains using many different research tools. In this paper, various research methods that have been introduced, developed, and utilized by neuroscientists in human creativity research studies are reviewed and discussed. This article will review the existing literature investigating the neural substrates of creativity in the human brain and discuss recent advances and new findings on underlying cognitive processes of human creativity. This review will particularly focus on how unique strengths and features of different research methods contribute to new discoveries that improve our understanding of human creativity by providing examples of research studies that utilized each of the research techniques.

Keywords: Creativity, Standardized Psychometric Test, Neuroimaging, Divergent Thinking

1. Introduction

Almost everything that surrounds us results from human creativity. Creativity enables us to visualize what that does not exist in the world and changes the way we behave and think in many significant ways. Because of the abstract and multiform ways that creativity has been defined, it is now widely considered by educators, psychologists, and business executives as the highest expression of learning, self-actualization, and a critical feature of leadership (Gabora, 2010). Moreover, our professional and personal success increasingly depends on it, as technology takes over other routine jobs.

Despite its essential roles in various activities, including literature, arts, technologies, and sciences, it is largely unknown how the human brain supports creativity. Because of its abstract and multiform definitions that likely involve many other mental processes, identifying neural mechanisms of human creativity is extremely challenging. One intriguing way to investigate the brain mechanisms underlying creativity is to dissect the brains of people who were extraordinarily creative during their lifetimes. For example, scientists found that Einstein's brain had (1) a partially absent Sylvian fissure, which may have resulted in facilitated communication between different brain areas, and (2) a high ratio of glial cells to neurons in the brain parts, such as prefrontal cortex, associated with planning, attention, and memory and left inferior parietal cortex, engaged in

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synthesizing information from other brain areas (Witelson et al., 1999). Visual illustrations of Sylvian fissure (shown in red), prefrontal cortex (blue circle), and inferior parietal cortex (green circle) are shown in Figure 1.

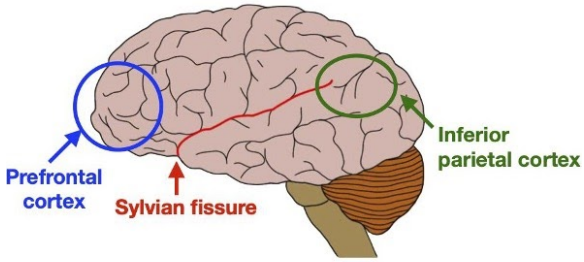

Figure 1. Parts of the brain potentially related to creative processing, including the prefrontal cortex, sylvian fissure, and inferior parietal cortex. Imagery from https://www.neuroscientificallychallenged.com/glossary/sylvian-fissure.

Recently, neuroscientists and psychologists define creativity as the human ability to formulate novel, practical, less-structured, and innovative ideas. Many attempts are being made to elucidate how we utilize our creative brains to morph the world around us into a more visionary and diverse place. Research methods, including neuroimaging techniques and standardized psychometric tests (Villarreal et al., 2013), are employed in neuroscientific and psychological studies to obtain a comprehensive understanding of brain functions underlying human creativity. Evidence suggests that divergent thinking is a central component of creativity (Kaufman et al., 2008). Therefore, the current review paper also includes research papers that specifically investigated and discussed divergent thinking.

2. Methods

For this review paper, articles were selected after searching for the databases available in Google Scholar (https://scholar.google.com/) and Pubmed (https://pubmed.ncbi.nlm.nih.gov/). The following keywords were used to determine relevant articles for this review paper: creativity, creative, divergent thinking, creative processing, research methods, neuroimaging, behavioral test, and psychometric tests, either separately or in combinations of some among them. As a result, 50 papers were available to be retrieved as full texts. From the articles retrieved, only the research papers published in the years from 1990 to the present were selected, eliminating 11 papers. Then the suitability was assessed for the current review paper based on the selection criteria below. First, all papers had to be written in or translated into English and already published in peer-reviewed journals rather than preprints. Also, any papers covered in this review had to include the keywords of creativity (or creative) or divergent thinking in the article in some significant way. For example, the keywords had to be included in the abstract or title or frequently mentioned in the main text. This criterion was to ensure that the included research papers were focused on creative thinking and behavior. Six more articles were then discarded because they only mentioned the keywords briefly or covered the keywords as secondary factors. In addition, all studies had to include novel empirical tests of creativity or divergent thinking using either neuroimaging techniques or behavioral, psychometric tests. As a result, 33 different articles that satisfied all the criteria remained available and suitable for this review paper once duplicates of articles had been spotted in each database.

3. Results

3. 1 Psychometric standardized tests

Since qualitative approaches have been traditionally used to investigate creativity, it has been challenging to generalize results from a group of individuals and compare between groups. Such a challenge has led to the development of test metrics that can be standardized and quantified for evaluating individuals' abilities of creative thinking. In typical psychometric standardized tests, individual subjects receive either questionnaires or simple behavioral tasks. Researchers then evaluate the subjects' responses based on standardized scoring systems. Creativity scores obtained from individual subjects can be compared across individuals to draw meaningful and generalizable conclusions. Some of the standardized measures for creativity research that are most commonly used are outlined below. Their order is based roughly on their prevalence in testing

of divergent thinking, with the Torrance Tests of Creative Thinking being the most popular test.

Torrance Tests of Creative Thinking (TTCT): The TTCT provides a useful tool that can evaluate an individual subject's creative potential (Humble et al., 2018; Runco et al., 2010; Zabelina & Robinson, 2010). TTCT has both verbal and non-verbal components with many different tasks. Consisting of multiple, independent tasks, TTCT evaluates fluency, flexibility, originality, and elaboration. Fluency is defined as the total number of interpretable, meaningful, and relevant ideas generated in response to the stimulus; flexibility is calculated as the number of different categories of relevant responses; originality is measured by comparing a subject's response to the total amount of responses from all of the people given the test (defined as "statistical rarity" of the responses); and elaboration is defined as the amount of detail in subjects' responses. For example, in one verbal task using verbal stimuli (termed as consequences task), subjects are provided with three improbable situations then asked to imagine and list out the consequences of the situations. In a product improvement task, another verbal task that uses non-verbal stimuli, subjects are given common objects (e.g., toys) and required to think of as many improvements as they can for the objects (e.g., making toys more fun to play with). They are also asked to think of unusual uses of the objects in this task. The non-verbal tasks (also termed as figural tasks) evaluate a subject's originality and elaboration potential, picture completion, fluency, and flexibility. An incomplete figures task uses a white paper on which an area of fifty four square inches is divided into ten squares, each containing a different stimulus figure. Subjects are asked to sketch or design novel objects by adding as many lines as they would like to the ten figures (see Figure 2 for an example). Scores from individual sections of the test are added to obtain the general score for the degree of creative thoughts.

Wallach-Kogan Test of Creative Thinking (WKCT): In the WKCT (Wallach & Kogan, 1965; Wallach & Wing, 1969; Wallach, 1970), subjects are asked to come up with as many possible items in a general group as they can. Some examples of general items include *round things, things with wheels,* or *things that make noise.* Just as in TTCT, subjects' responses are scored using the four components: fluency, flexibility, originality, and elaboration.

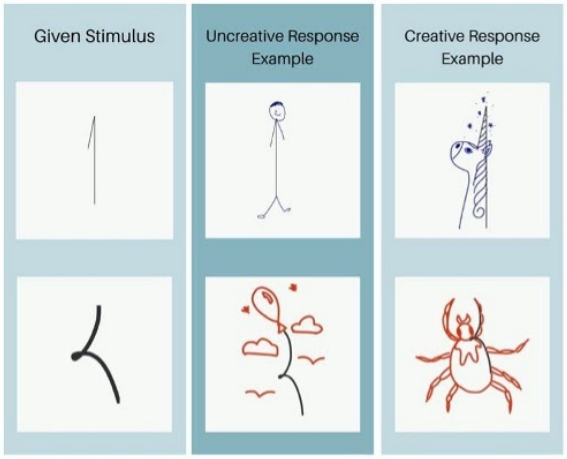

Figure 2. Examples of creative and uncreative responses in the TTCT drawing test. Participants are provided a stimulus over which they must draw a new figure during a given time interval.

Guilford's Alternative Uses Test 1967 (AUT): In this test, subjects are asked to list possible uses for a common item, such as a newspaper, a sponge, or a brick. Scoring comprises the same four basic components: originality, fluency, flexibility, and elaboration (Gilhooly et al., 2007; Olteteanu & Falomir, 2016; Vartanian et al., 2019).

Remote Associate Test (RAT): The RAT is a test of creative potential based on associations and convergence, which has been utilized a wide range of cognitive research studies on creativity (Mikulincer & Sheffi, 2000; Storm, et al., 2011; Vohs & Heatherton, 2001). In the RAT, each question consists of three common stimulus words that appear to be unrelated. Subjects are then asked to think of a fourth word that is somehow related to each of the first three words. The test typically consists of thirty to forty questions, and scores are calculated based on the number of correct items.

3.2 Brain imaging techniques

Recent advances in science and technology allow researchers to investigate the brain

mechanisms underlying human creativity by examining patterns of brain activity when people are engaged in creative activities. The power of neuroimaging research on human creativity is that it can provide measurements of neural activities that are directly associated with creative thinking and behaviors of individuals. Neuroimaging research typically involves participants taking some form of creativity test or thinking task while measuring brain activity. Below, some neuroimaging techniques that are commonly used in creativity research are discussed, with fMRI typically being the most common and PET being the least common.

Functional Magnetic Resonance Imaging (fMRI): When neurons become active, blood supply to the active brain area increases, leading to a change in the relative concentration of oxyhemoglobin and deoxyhemoglobin. fMRI uses a strong magnetic field to measure these relative changes in concentration of oxyhemoglobin and deoxyhemoglobin by using a technique known as blood oxygen level dependent signal (BOLD). The brain areas with increased neuronal activities require more blood, consequently, increasing the BOLD signal. fMRI can capture these BOLD signals, providing impressive images about patterns of brain activations with very precise maps of the brain regions (Gonen-Yaacovi et al., 2013; Logothetis et al., 2001).

There are several fMRI studies in brain mechanisms of creativity using particular cognitive tasks such as divergent thinking, story generation, AUT, novel metaphors test, and three-word remote associates test (Beaty et al., 2014; Fink et al., 2009; Gold et al., 2012; Kleibeuker et al., 2013; Takeuchi et al., 2011). Most of these cognitive tasks are either directly or indirectly relevant to testing schemes of various psychometric standardized tests, outlined above. In these fMRI studies, subjects are positioned in a fMRI scanner (typically in a supine position; Figure 3A) and asked to perform experimental tasks. In one interesting fMRI study, researchers recorded jazz musicians' brains while they created musical improvisations using a musical instrument specially made to be used within the fMRI scanner (Limb & Braun, 2008). The researchers discovered salient brain activations during musical improvisation in the medial prefrontal cortex, which is known to be also involved in self-motivated behaviors, independent of external stimuli. They also discovered concurrent deactivation in other areas of the prefrontal cortex, which is known to be involved in focused attention. It has been known that the medial prefrontal cortex may be a major constituent of a "default" system that remains active at rest and may be related to altered states of consciousness such as hypnosis, meditation, and daydreaming (Andrews-Hanna et al., 2014; Konjedi & Maleeh, 2017). In another fMRI study, subjects performed creative story generation and found significant brain activations within bilateral medial frontal gyri and the left anterior cingulate (Howard-Jones et al., 2005). Therefore, based on the new discoveries, researchers suggest that the creative production of novel materials may occur outside of conscious awareness and volitional control, possibly mediated by activation of the medial prefrontal cortex. They conclude that improvised creativity may be in a mode of defocused, free-floating attention that allows for spontaneous associations or insights.

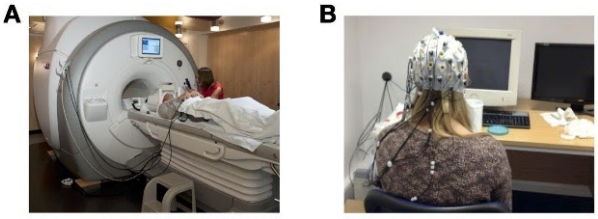

Figure 3. Examples of fMRI(A) and EEG testing(B). Image 3A provided by https://news.stanford.edu/news/2013/march/brain-imaging-inaccuracies-030713.html. Image 3B provided by https:/bournemouthbrainandcognitionlab.wordpress.com/want-to-participate-in-an-eeg-study/taking-part-in-an-eeg-s tudy/.

Electroencephalography (EEG): Neurons are densely interconnected via synapses, and any synaptic activity results in a subtle electrical impulse, termed as a postsynaptic potential. Because the signal is extremely weak, it is almost impossible to reliably detect the burst of a single neuron without direct single-cell recording via electrodes implanted in the brain. However, when

thousands of neurons fire in sync (e.g., occur in a similar location or a similar rhythm), an electrical field adds up to be strong enough to be measured on the head surface. EEG is a physiological measuring technique that records the electrical activity generated by neural activities of the brain via electrodes placed on the scalp surface, which is safe and non-invasive (Figure 3B). Typically, electrodes are attached to adjustable, elastic caps similar to bathing caps to ensure that the data can be easily collected from the whole scalp. Because EEG provides excellent time resolution, it allows researchers to detect activity within cortical areas even within sub-seconds, which makes EEG highly suitable for studying temporal characteristics of the brain mechanisms of creative thinking and behaviors.

Various behavioral tasks have been utilized in EEG research studies, including insight problems from the RAT, stories from GDTT, Divergent Thinking test, and imaginary drawing while looking at a while wall (Benedek et al., 2011; Jauk et al., 2012; Krug et al., 2003). In one EEG study, for example, subjects performed Guilford's alternate uses task (AUT) as well as basic word association tasks while EEG was recorded. In this study, subjects were instructed to find either the most common solution (convergent condition) or the most uncommon solution (divergent condition). Researchers found that when subjects were thinking of the most uncommon solution (divergent processing), their EEG measurements showed stronger and more meaningful synchronization of neurons in an alpha rhythm (the normal electrical activity of the brain consisting of neural waves with a frequency of 8 to 13 hertz), compared to when they were looking for the most common solutions. The researchers concluded that a group of neurons' synchronization (e.g., being active and fired together) in an alpha rhythm could be associated with divergent cognitive processing. Another group of researchers also found that divergent thinking tasks produced decreased synchrony in a beta rhythm (neural waves with a frequency range of between 12.5 to 30 hertz), concurrent with increased alpha rhythm synchrony in the frontal area. It is known that alpha rhythm synchrony is related to loosened control (e.g., inhibitory function) and minimal neural arousal, whereas beta rhythm is associated with synchrony controlled cognitive processing and active thinking (Neubauer et al., 2006). Some other researchers found that increased power in the frontal cortex and increased desynchronization over the posterior cortex is associated with the performance during a verbal insight task (Razumnikova et al., 2007). One EEG study also directly compared 25 gifted individuals and 25 age-matched controls with the same age were compared and reported that the gifted subjects showed increased information transfer within left posterior brain regions compared to controls (Jin et al., 2006). Together, EEG studies present insightful findings on creative thinking and processing, providing converging evidence that creative thinking (or divergent thinking) occurs in a mode of loosened cognitive control without mentally demanding, focused attention.

Positron Emission Tomography (PET): When neurons are firing, the neural activation elevates and leads to an increased blood supply as well as increased oxygen level. PET introduces a "radioactive tracer" into the bloodstream and measures the differences in regional cerebral blood supply (rCBF) to quantify local neuronal activity (Vanitha, 2011). Measurement of rCBF is based on the fact that where there is more blood flow to a specific location of the brain, more radioactive tracer accumulates in the brain tissue, resulting in greater radiation emissions from the active brain areas. Although not many, there have been a few creativity tests conducted using PET. One PET study included Creative Functioning Test in which subjects create a story using either easy or hard words provided by researchers or verbal insight tasks (Starchenko et al., 2003). These studies found that the creative thinking process activated the left parietal cortex and the cingulate gyrus. In another PET study by the same group of researchers instructed subjects to perform verbal creativity tasks and found brain activations in the left parietotemporal brain regions (Bechtereva et al., 2004).

Employing the optimal neuroimaging technique is critical in neuroscientific research, as each technique’s use is dependent on the goal of the

research. For example, EEG is optimal for examining the temporal characteristics of brain function, such as how fast the brain processes information, since EEG has high-quality great temporal resolution compared to PET and fMRI. However, if the goal of the research study is to understand where in the brain certain information is processed, then fMRI would be ideal because of its spatial resolution. Ergo, each neuroimaging technique has different applications in understanding the role of creative thought in the brain.

4. Discussion

It is perhaps our creativity that makes humans unique and distinguished from other species on our planet. There are several cognitive neuroscience methods that allow creativity researchers to investigate the brain mechanisms of creativity, and this review paper has introduced some of them that provided new and informative discoveries. Brain imaging techniques greatly contribute to a better understanding of how humans come up with novel and creative thoughts and our capacity to identify the areas of the brain associated with creative processing. Although many studies shed light on the areas of the brain involved in creative and divergent thinking, we know very little about the whole picture of it. Moreover, many controversies still remain to be resolved by neuroscience researchers. First of all, there are controversies regarding sex differences in creative thinking (Abraham et al., 2014; He & Wong, 2011; Shen et al., 2015). Although it is commonly thought and widely accepted that males and females have relatively little difference in their creative abilities, some other studies also have reported direct conflicts in their findings (e.g., Krumm et al., 2016). For example, researchers (He & Wong, 2011) found that males performed significantly better than females on some cognitive tests that require creative thinking such as the continuation, boundary-breaking, and unconventionality tests, suggesting that males show a higher degree of creative thinking. In contrast, other researchers also reported that DT skill was significantly better in females than males (Kuhn & Holling, 2009). While most of the previous research reported small differences in performance between sexes, these differences should not guide education approaches on male-or-female class basis. Since these results are considered statistically insignificant, implementing them in schools will inevitably justify a degree of gender inequality within academia, with one sex being seen as more or less creative.

Appropriately identifying whether the observed sex differences reflect truth is important for fostering creativity and educating children, improving school curriculums, and maximizing students' creative output and innovative success. Future studies should determine sex differences in creative thinking and behaviors in various domains.

Another controversial issue on creativity is whether human creativity is domain-specific (e.g., creativity in one domain is not expected to enhance creativity in another domain) or domain-general. Support for domain-specificity comes from findings that expertise on one creative endeavor is only rarely associated with expertise on another (Baer, 2010). For example, gifted scientists who are famous for creative thoughts and innovative ideas do not necessarily become great artists or musicians. On the contrary, some psychologists tend to view creativity as more domain-general because the process of associative and divergent thinking can produce metaphors across different domains (e.g., music and visual art). The view of domain-generality of creativity is also supported by self-report scales, creativity checklists, and other psychometric or personality data (Plucker, 1998). There are also experimental reports that, when subjects recognize someone's creative style in one domain (e.g., creative writing), it is likely that works by that individual in another domain (e.g., art) are also recognized by subjects (Gabora et al., 2012). The truth likely lies somewhere between the extremes: creativity in one domain may greatly help but not completely guarantee creativity in another. Future studies are needed to explore this issue further.

Improving divergent thinking in regards to education via pedagogy is a growing topic within neuroscience research. One method showing particular promise is Drama-based pedagogy, or

drama pedagogy training (DPT). This teaching method emphasizes the use of drama and active learning to better teach students in a range of fields, often playing on elements of improvisation and roleplay. The effects of DPT on creative thinking seemingly vary with age, with studies on preschool-age students finding that DPT had a large effect on creativity whereas studies in 9-10 year olds more typically finding moderate effects on creativity (Yeh & Li, 2008; Hui & Lau, 2006). However, further study on DPT is necessary to streamline its application, as current DPT has no set standards and often misreported protocol that may lead to significant variation in present research. Pursuing applied research in DPT and other pedagogical methods for fostering creativity can assist students in growing their abilities to perform high-level creative tasks and thinking skills.

5. Conclusion

This paper reviewed some of the psychometric standardized tests and neuroimaging research methods that are commonly utilized in research studies on human creativity. When combined, psychometric tests and neuroimaging studies can provide straightforward and quantifiable data for comprehensive pictures of human creativity and brain mechanisms of creative processing and innovative ideas. Neuroscience research in creativity is a relatively new and exciting area that can be closely linked to many different fields, including education, philosophy, business, as well as computational and mathematical modeling. Promising areas for further research in creativity include computational modeling of neuroimaging data as well as genetic and environmental influences on creativity.

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Isolation and Inhibition of Psychrotrophic Fungi in Dairy Products

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Abstract

The growth of fungi is inevitable in dairy products. They are common contaminants and are often responsible for differences in the color and flavor of a dairy product. The control of fungal spoilage is also a major concern for scientists and food producers as the growth of fungi results in great food waste and economic losses. Though most fungi are harmless, certain fungi produce mycotoxins which can also contribute to various health problems and illnesses. Therefore, using food preservatives is essential in our foods to avoid contamination. However, the chemical preservatives have proven to be harmful for human health, thus the need for using safe, natural preservatives has increased. Essential oils of aromatic plants have been recently used as antimicrobials and food preservatives. This study was conducted to isolate and identify some of the psychrotrophic fungi that contaminate dairy products in our refrigerators using morphological and molecular tools. In order to control the fungal growth, we studied the effect of essential oils: eugenol and isoeugenol, as well as the temperature effect on two of the isolated fungal species using a radial growth method. Our experiments revealed that isoeugenol had a better inhibition effect on the two tested fungi:*Penicillium paneum* and *Penicillium verrucosum*. The essential oils as well as lower temperature, 15°C were good parameters to inhibit/reduce the fungal growth.

Keywords: Psychrotrophic Fungi, Penicillium, Essential Oils, Mycotoxins, Refrigerators

1. Introduction

Food spoilage during storage is a major environmental problem and is a great concern for food industries. There is about 1.3 billion tons of food that is wasted every year from initial agricultural production down to the consumer (Gustavsson, 2011). Fungi are common contaminants of dairy products stored in refrigerators. They are responsible for visible or non-visible defects. It can lead to a significant food waste as well as important economic losses (Garnier, et al., 2017). The group of fungi that is capable of growing at low temperatures is called Psychrotrophs (Basavabharati and Prabha, 2015). Those fungi have the ability to use many substrates including carbohydrates, organic acids, proteins, and lipids, which are present in milk and its products in order to grow (Veld, 1996).

The predominant psychrotrophic mold species isolated from refrigerated foods were *Penicillium* (49%) and *Aspergillus* (38%) (Torrey and Marth, 1977). The psychrotrophic mold species of *Penicillium* were also predominant in fermented dairy products (Bullerman, 1981). The refrigerated storage and pH of traditional dairy products helps the growth of psychrotrophic molds which may have harmful effects on humans and animals, as well as cause defects in products (Basavabharati and Prabha, 2015).

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Most of the psychrotrophic molds produce proteinases and lipases that change the composition of milk constituents leading to defects that in turn cause economic loss to the producers.

Some of these molds are mycotoxin producers. Mycotoxins are secondary metabolites that are produced by fungi (Del Palacio, et al., 2016). Most mycotoxins are chemically stable and survive food processing, which is a concern to human health. In cheese, the most hazardous mycotoxins are ochratoxin A and Aflatoxin M1. They are produced by fungal species either via direct cheese contamination or indirect contamination (where the milk used to make the cheese was contaminated) (Hymery, et al., 2014). A case has been reported of a man with a history of poorly controlled diabetes, acute myelogenous leukemia, and prolonged neutropenia who presented a unilateral headache, fever, nausea, and vomiting. A sinus culture yielded *Mucor circinelloides* and the suspected source of infection was contaminated Greek yogurt, which he consumed several days before developing these symptoms (Lazar, et al., 2014).

Scientists are looking for efficient solutions to prevent and/or limit fungal spoilage in dairy products. Different traditional technologies are used to control contaminants such as air treatment, cleaning and disinfection procedures, heat treatment, and water activity reduction during refrigeration (Altunatmaz, 2012). Moreover, chemical preservatives such as benzoate and sorbate are used to avoid fungal spoilage (Silva and Ladon, 2016). However, adverse side effects have been reported as a result of using these chemical preservatives. Benzoates have been suspected to cause allergies, asthma, and skin rashes. Also, sorbates were reported to cause urticaria and contact dermatitis (Kinderlerer and Hatton, 1990).

Therefore, using natural safe preservatives such as essential oils to prevent food contamination is being considered in the food industry (Nasery, et al., 2016). Aromatic and medicinal plants produce essential oils in the form of secondary metabolites (Pandey, et al., 2017). These compounds have been tested to as antibacterial and antifungal agents against some microorganisms. Eugenol is a phenylpropanoid and is extracted from different natural sources such as clove trees (*Syzygium Aromaticum*). It has antimicrobial activity against microorganisms such as bacteria and fungi. Eugenol and its derivatives were studied on *Botrytis cinerea* and showed a good inhibitory effect on this fungus (Olea et al., 2019). However, these oils have not been studied on *Penicillium paneum* and *Penicillium verrucosum.*

The information provided in the previous background explains how important it is to prevent fungal contamination to ensure a healthy food delivered to the consumers. Besides, there is a high demand to use natural compounds rather than using synthetic ones. Therefore, in this study, different fungal species were isolated from various dairy products. *Penicillium paneum* and *Penicillium verrucosum* isolated from Mozzarella and Havarti cheese, were selected to study the effect of the essential oil, eugenol, and its derivative, isoeugenol as well as the effect of temperature on their radial growth.

2. Methods

Ten dairy samples were collected from home refrigerators including milk, sour cream, havarti cheese, yogurt, pepper jack cheese, sharp cheddar cheese, medium cheddar cheese, roomy cheese, and butter. All samples had already shown a significant fungal growth.

Czapek-Dox agar media was used as a general culture media that allowed the growth of most fungi. Rose Bengal dye and the antibiotic (chloramphenicol) were added to the media to suppress bacterial growth. Chilvers et al. (1999) proved that the combination of Chloramphenicol and Rose Bengal Dye has been scientifically proven to inhibit bacterial growth along with the presence of light. Chloramphenicol itself has also been deemed as a durable antibiotic due to its ability to withstand heat, which allows for it to be added to any medium before sterilization (King, et al., 1979). In order to prepare food homogenates, 5 grams of each rotten dairy sample was taken, and mixed with 10 ml of deionized water. The samples were then crushed with a mortar and pestle and transferred into an Eppendorf tube which was then vortexed. 200 µl of the fungi mixture was added to each set petri dish and

incubated at 20 degrees Celsius for eight days. Duplicate plates were prepared from each isolate. Afterward, an additional direct inoculation of the contaminated food samples was done for each dairy sample. So, three plates from each food sample were prepared.

On the ninth day of inoculation, fungi were taken from each petri dish and re-inoculated into a new set of petri dishes, all without Rose Bengal dye and chloramphenicol. The new set of petri dishes were then incubated at 20°C for ten days. The reinoculation process was repeated a total of 3 times to be ready for the identification.

2.1 Fungal Isolates Identification

Both macroscopic and microscopic examination of the isolated fungi were done using the identification books "Identifying fungi: A clinical laboratory handbook" (St-Germain and Summerbell, 2011), and "Fungi and Food Spoilage" (Pitt and Hocking, 2009). We also did molecular identification to confirm our isolated species.

2.2 Molecular Method

Mycelia was scraped from each of the eight samples' petri dishes and mixed with liquid nitrogen until it became a fine powder. The genomic DNA was then extracted from each powder using the procedure outlined in the Zymo Quick-DNA™ Fungal/Bacterial Miniprep Kit (Catalog No. D6005). The DNA concentration of each sample was measured using a fluorometer.

The internal transcribed spacer region "ITS" were amplified. The ITS region is the most recommended universal fungal barcode sequence that is used because of its highest probability of successful identification of fungi. The DreamTaq Hot Start PCR Master Mix (2X) with primers ITS1F and ITS4 (forward and reverse primers) were used (White, et al., 1990). 25µl of PCR mixture was mixed with 2.5 µl of each of the two primers: ITS1F and ITS4, 10µl of our sample's DNA, and 10µl of nuclease free water. The eight samples were placed in Perkin Elmer Cetus 480 Variable Temperature DNA Thermal Cycler at fixed intervals: 95°for 5 minutes, then a repetition of 35 cycles for 94°C for 30 seconds, 52°C for 30 seconds, 72°C for 1 minute, followed by 72°C for 8 minutes, and then a 5-minute hold at 4°C.

Detection of PCR-amplified products was performed by electrophoresis on 1 % ethidium bromide-stained agarose gel. Agar gel was made with 25 mL of water, 0.25 g of biology-grade agar powder and 12.5 µl of ethidium bromide. The water and agar powder were microwaved on high for approximately 75 seconds on before mixed with the ethidium bromide and set to dry in a mold. The PCR products had left to cool, then 8.3µl of loading dye was added to each 10 µl of dyed sample (including a DNA ladder) which were then put into each well. The machine ran at 110 volts for around 20 minutes, until the gel traversed across the plate. The agar plate was removed and was put on top of a UV light, where the bands could be seen. The base pairs for each sample came to 500 base pairs for pepper jack, sharp cheddar, butter, milk, and Havarti cheese, and 850 base pairs for sour cream and roomy cheese.

PCR products were purified using the procedure provided with the kit "DNA Clean & ConcentratorTM-5". 10µl of each DNA sample was taken and added to 50 µl of DNA binding buffer as a 5:1 ratio had to be maintained. The mixture was put into a column with a collection tube underneath and centrifuged. 200 µl of DNA wash buffer was added and followed by centrifuging. 15 µl of DNA elution buffer was then added and stored in an Eppendorf tube. The concentrations of each sample were taken again using fluorometer. The purified PCR products were sent to Elim Biopharmaceuticals to do Sanger sequencing. The consensus sequences of the ITS region were submitted for a BLAST search using the NCBI GenBank database to obtain species-level information.

2.3 Essential Oils and Temperature Effect

The antifungal activity of Eugenol and Isoeugenol was tested on two of the isolated fungal species: *Penicillium paneum* and *Penicillium verrucosum* which were isolated from mozzarella cheese and Havarti cheese respectively. Eugenol (concentrations applied were: 250, 500, and 750 ppm) and isoeugenol (concentrations applied were: 250, 500 and 750 ppm) were dissolved in 5% of Dimethyl Sulfoxide solution (DMSO). CDA media was used as a growth medium

for radial growth measurements to test the inhibitory effect of eugenol and isoeugenol essential oils at three different temperatures: 15°C, 20°C, and 25°C. They were added to the medium after sterilization and daily measurements were taken for 7 days. Three readings were recorded daily for each treatment (n=3).

3. Results

Out of the ten collected dairy samples, eight different species of *Penicillium* were identified. They were as follows: Yogurt - *Penicillium decumbens*, Butter - *Penicillium brevicompactum*, Cheddar cheese (Sharp + Medium) -*Penicillium commune*, Roomy cheese and pepper jack - *Penicillium crustosum*, Sour cream - *Penicillium solitum*, Milk - *Penicillium chrysogenum*, Havarti cheese - *Penicillium verrucosum*, Mozzarella - *Penicillium paneum*. Most of the *Penicillium* spp. are known as mycotoxin-producers, which are hazardous to humans.

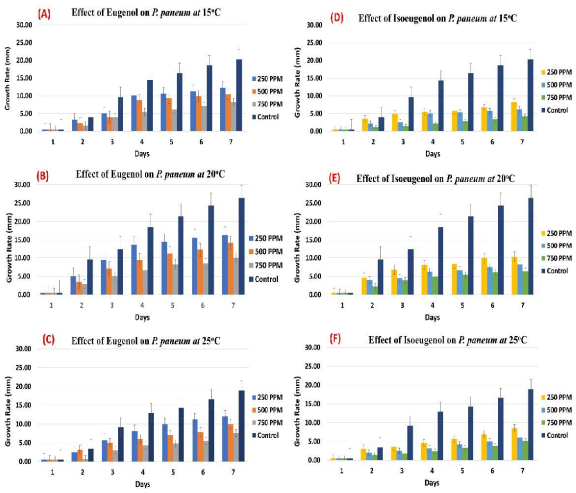

Figure 1: Growth Rate of *P. paneum* when in contact with (A) Eugenol at 15°C, (B) Eugenol at 20°C, (C) Eugenol at 25°C, (D) Isoeugenol at 15°C, (E) Isoeugenol at 20°C, and (F) Isoeugenol at 25°C, compared with the control for 7 days. Legend (top to bottom) for subgraphs A, B, and C: Eugenol concentrations of 250 ppm, 500 ppm, 750 ppm and control plates (no oil). For subgraphs D, E, and F: Isoeugenol concentrations of 250 ppm, 500 ppm, 750 ppm and control plates (no oil). The data are expressed as the mean ± SEM (n = 3)

Our experiment with the essential oils and the temperature effect showed that isoeugenol was better than eugenol in terms of inhibiting the radial growth of the selected fungi, *Penicillium paneum* and *Penicillium verrucosum* (Figure 1 & Figure 2). Moreover, 20°C was the optimum growth temperature for both fungal species. In the figures 1 and 2, our control samples (no oils added) showed that both *P. paneum* and *P. verrucosum* grew optimally at this temperature, while at the other two temperatures, their growth was better at 15°C than at 25°C. The percentage of inhibition for each treatment was calculated as follows:

$$\% \text{ inhibition} = \frac{\textit{Growth rate of the control} - \textit{Growth of the treated fungus with oil}}{\textit{Growth rate of the control}} \times 100$$

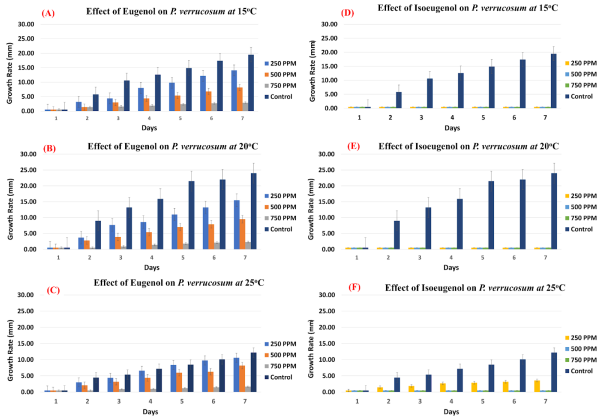

Figure 2: Growth Rate of *P. verrucosum* when in contact with (A) Eugenol at 15°C, (B) Eugenol at 20°C, (C) Eugenol at 25°C, (D) Isoeugenol at 15°C, (E) Isoeugenol at 20°C, and (F) Isoeugenol at 25°C, compared with the control for 7 days. Legend (top to bottom) for subgraphs A, B, and C: Eugenol concentrations of 250 ppm, 500 ppm, 750 ppm and control plates (no oil). For subgraphs D, E, and F: Isoeugenol concentrations of 250 ppm, 500 ppm, 750 ppm and control plates (no oil). The data are expressed as the mean ± SEM (n = 3)

The experiment revealed that at concentration 750 ppm, the highest inhibition percentage of isoeugenol on *P. paneum* was 79.3% at 15°C (Figure 3). At the same temperature, the eugenol also appeared to inhibit the *P. paneum* growth by a maximum inhibition 59.6% at 750 ppm (Figure 3). The radial

growth decreased as the concentration of the essential oils increased (Figure 3 & Figure 4).

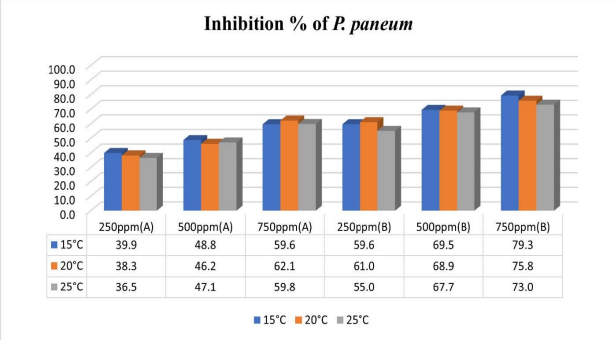

Figure 3: Percent inhibition of *P. paneum* when exposed to 250 ppm (A), 500 ppm (A), and 750 ppm (A) of eugenol at 15°C, 20°C, and 25°C and 250 ppm (B), 500 ppm (B), and 750 ppm (B) of isoeugenol at 15°C, 20°C, and 25°C.

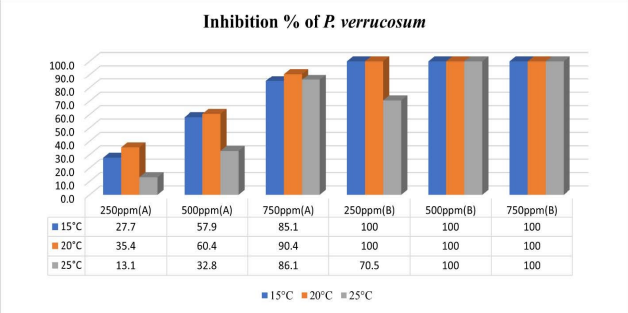

Figure 4: Percent inhibition of *P. verrucosum* when exposed to 250 ppm (A), 500 ppm (A), and 750 ppm (A) of eugenol at 15°C, 20°C, and 25°C and 250 ppm (B), 500 ppm (B), and 750 ppm (B) of isoeugenol at 15°C, 20°C, and 25°C

However, the inhibition percentage of eugenol on *P. verrucosum* was higher at 20°C (Figure 4). The isoeugenol at the tested concentrations was able to inhibit the growth by 100% for *P. verrucosum* except at 25°C at 250 ppm concentration as shown in Figure 2-D, E & F and the growth was still inhibited by 70.5% (Figure 4). However, isoeugenol could not completely stop the growth of *P. paneum* as depicted in figure 1-D, E & F. The inhibition percentage was 61%, 69.5%, and 79.3% at 250 ppm, 500 ppm, and 750 ppm respectively on the growth rate regardless of the temperature (Figure 3). Although the growth of *P. paneum* did not stop at all concentrations of eugenol and isoeugenol, the fungal growth rate decreased as the concentration increased (Figure 1). Our work also revealed that both oils had greater inhibitory effect on *P. verrucosum* than their effects on *P. paneum*. The *P. verrucosum* growth was inhibited by a maximum of 90.4% at 750 ppm eugenol (Figure4); but for *P. paneum*, the inhibition rate was only 62.1% at the same concentration (Figure 3). The highest percentage of inhibition by the added essential oils were observed at temperatures 15°C and 20°C. Although both fungi did not grow well at 25°C without any oil added, and the growth rate of the control at 25°C was less compared to the growth at the other two temperatures, the oils inhibitory effect was less at this temperature.

4. Discussion

Fungi are common contaminants of dairy products, which provide a favorable environment for their growth as they are responsible for visible or non-visible defects, such as off odor and flavor (Garnier et al., 2017). Although temperature is one of the major controlling factors of food quality and safety because of its influence on microbial growth rates, fungal spoilage is still an issue for dairy manufacturers. The reason behind this fact is that psychrotrophic microorganisms have the ability to grow at normal refrigeration temperatures (Altunatmaz, 2012). Furthermore, fungi may originate from milk or may be introduced during cheesemaking either from the environment or are deliberately inoculated using commercial ripening cultures (Hymery, et al., 2014).

Eight different *Penicillium spp.* were identified in all our isolates using morphological and molecular methods. *Penicillium commune* was isolated from sharp and medium cheddar cheese. This fungus belongs to the Ascomycota phylum, which has been often isolated from hard or semi-hard cheeses (Garnier, et al., 2017). It is also well known for being one of the most common fungal spoilage molds of cheese, which produces two neurotoxins, penitrem A and roquefortine, and many mycotoxins, such as cyclopiazonic acid and regulovasine A and B (Gqalen, et al., 2001). Garnier et al. (2017) reported that *Penicillium commune,* along with many other fungi like *Penicillium solitum*, *Penicillium*

crustosum, *Penicillium verrucosum*, *P. chrysogenum*, *Penicillium nalgiovense*, and *Penicillium griseofulvum* were isolated from hard and semi-hard cheeses but were also found in many other milk products such as butter, yogurt, and milk.

Within our study *Penicillium brevicompactum* was isolated from butter. This fungus was previously isolated from fruits and can grow between -2°C and 30°C with an optimum near 23°C (Pitt and Hocking, 2009). In the same study in which *P. commune* was isolated from cheese (Garnier, et al., 2017), *P. brevicompactum* was similarly isolated from various hard and semi-hard cheeses. *P. brevicompactum* produces the weak mycotoxin mycophenolic acid which is not a concern in foods. *Penicillium solitum* was isolated from sour cream, but Yin et al. (2016) asserted that this fungus is often found in fruits and is usually responsible for the decay of apples and pears (pome fruits). Furthermore, *P. Solitum* was the most dominant species isolated from Italian hard cheese (Decontardi, et al., 2017). *P. solitum* is one of the less important species of fungal organisms responsible for food decay.

In the mozzarella cheese, *Penicillium paneum* was identified. According to the article from American Society for Microbiology, *P. paneum* is a common contaminant found in cereal grains (Chitarra, et al., 2004). This fungus can grow at low temperatures, low pH, high levels of carbon dioxide, and in acidic conditions. However, in this research, it was isolated from a sample of a dairy product. So, as a result *P. paneum* can grow both in wheat products as well as dairy products in many different conditions. *P. paneum* is known to produce patulin, which is mutagenic, immunotoxic, and neurotoxic (Cole and Cox, 1981).

Penicillium Crustosum was isolated from roomy cheese and pepper jack cheese in our experiment. This fungus is usually reported causing blue mold on pome fruits but is also found on cheese and nuts. It produces many mycotoxins such as roquefortine C, terrestric acid, and penitrem A. According to Vico et al. (2014) *Penicillium Crustosum* was found on apples in Serbia However, in this research, *Penicillium Crustosum* was detected in dairy products, rather than fruits.

Another species of fungi obtained from yogurt was *Penicillium decumbens*. Liu et al. (2013) found this fungus in decayed straw-covered soil in China. In their experiment, it was used to produce industrial-scaled cellulase. However, Vadillo et al. (1987) reported *Penicillium spp*. in pasteurized milk, which is consistent with our results.

Penicillium verrucosum was isolated from Havarti cheese. This fungus is known to grow between 0°C and 31°C with an optimum temperature around 20°C (Pitt and Hocking, 2009). Kure and Skare (2019) reported that *P. verrucosum* was found in hard, semi-hard and semi-soft cheeses from Greece, Denmark, and Spain. It produces intoxicating mycotoxins called ochratoxin A (OTA). This mycotoxin is an immunosuppressive and teratogenic (Pitt and Hocking, 2009). It has also been classified as genotoxic and a possible human carcinogen (Pfohl-Leszkowicz and Manderville, 2007). OTA production increases when temperatures are between 10°C and 21°C. Therefore, the presence of this fungus in our cheese is a huge concern for human health.

Penicillium chrysogenum was found in milk. This *Penicillium spp*. has been frequently observed in cheese from Denmark, USA, and Spain (kure and Skare, 2019). *Penicillium chrysogenum* is rarely pathogenic, but it was reported to cause health problems to people with weak immune systems (Adrian, et al., 2005). Moreover, *Penicillium chrysogenum*, *P. citrinum*, *P. commune*, *P. decumbens*, and *P. roqueforti* were also reported to be isolated from cheese surfaces stored at refrigeration temperature (6± 2°C) (Makki, 2019).

Three concentrations of the selected essential oils were used ranging from 250 ppm to 750 ppm of eugenol and its analogue, isoeugenol, because the US Food and Drug Administration (FDA) recommends using less than 1500 ppm of essential oil concentrations extracted from cloves. Although it has been approved for use in food as a safe food preservative, studies showed that clove oil is toxic to human cells if ingested in high concentrations. It has been shown to cause life-threatening complications such as acute respiratory distress syndrome and fulminant hepatic (liver) Failure (Kegley, et al., 2010). In our experiment, isoeugenol has shown better inhibitory effect on the two selected species,

Penicillium verrucosum, and *Penicillium paneum* (Figure1 & Figure 2). It completely suppressed the growth of *Penicillium verrucosum* at the three concentrations except at 250 ppm at 25°C (Figure2). For *Penicillium paneum* isoeugenol had a significantly greater effect than eugenol, but did not suppress the growth completely, or as much as it suppressed *Penicillium verrucosum* (Figure1). Isoeugenol and eugenol have been reported to have the ability to slow or stop the growth of fungi (Torrey and Marth, 1977). According to Hamini-Kadar et al. (2014), the eugenol has stopped the growth of *Fusarium redolens* and *Fusarium commune* at 500 ppm. However, in this research, the eugenol did not stop the *P. verrucosum* nor *P. Paneum* growth. 750 ppm inhibited the growth of *P. verrucosum* by a maximum of 90.4% (Figure4), while *P. paneum* growth was suppressed by 62.1% (Figure3). Šimović et al. (2014) used 250 ppm and 750 ppm of eugenol and carvacrol, which showed significant inhibitory effects against fungal pathogens, specifically *Aspergillus carbonarius* and *Penicillium roqueforti*. They incubated them at 15°C and 25°C and measured the growth rate for six days. They asserted that the eugenol had a synergy effect on the watermelon, because the inhibitory effect was greatest at 15°C. This is consistent with this study as the percentage of inhibition of eugenol and isoeugenol was the highest at 15°C by 59.6% and 79.3% respectively on *P. paneum* (Figure3). However, the greatest inhibition of eugenol on *P. verrucosum* was 90.4% at 20°C (Figure4). Our experiments suggest that isoeugenol is the best essential oil that can be used as an antifungal agent against the tested fungal species since it inhibited *P. verrucosum* by 100% and *P. paneum* by 79.3% at 750 ppm respectively. At lower temperature 15°C and 20°C, the percent of inhibition for both fungi were closer to or higher than that at 25°C (control temperature outside fridge). Therefore, using isoeugenol as a food additive for dairy products stored at refrigerators is recommended.

The antifungal mode of action of eugenol and its analogs needs further investigation, but it is known to affect cell proliferation. Using eugenol as an antimicrobial agent altered cell membrane and cell wall structures of proliferating *Saccharomyces cerevisiae* cells resulting in the release of cellular content (Bennis, et al., 200; Hyldgaard, et al., 2012; Maia da Silva, et al., 2018). One study evaluated the isoeugenol's antifungal action against *Candida spp*., and stated that isoeugenol inhibits H+-ATPase, which triggers intracellular acidification and cell membrane breakages (Bhatia, et al., 2012).

5. Conclusion

The purpose of our research was to identify different psychrotrophic fungi from dairy products. Eight different *Penicillium spp*. were isolated from different dairy products stored in refrigerators. The inhibitory effect of eugenol and its analogue, isoeugenol, was studied at different temperatures on the radial growth rate of *P. paneum*, and *P. verrucosum*, which were isolated from Mozzarella and Havarti cheese respectively. Isoeugenol had a greater effect of suppressing the fungal growth in comparison to eugenol at all the selected temperatures. *P. Paneum*, and *P. verrucosum* grew optimally at 20°C as they are considered psychrotrophic fungi. The essential oils were successfully able to lower the fungal growth at lower temperature 15°C and 20°C. In conclusion, isoeugenol is the best essential oil that can be used as an antifungal agent against the tested fungal species. Treating food with essential oils could be a viable solution to the spoilage problem of our dairy products, however further work is still needed to study a wider range of eugenol analogs on more fungal species. Food decay is an ever-growing issue in the food industry. Spoilage of our food with mycotoxin-producing fungi can cause serious health problems in humans. Therefore, using preservatives to avoid contamination and the eventual production of mycotoxins in our food is essential. Since synthetic preservatives have appeared to be harmful to human health and the environment, the use of natural and safer preservatives is currently recommended. Using essential oils of aromatic plants as preservatives is suggested to help prevent the deterioration of food.

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Disrupted to United: How Park Chung Hee's Authoritarian Rule Turned South Korea from a Falling Nation to an Economic Tiger

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Abstract

Due to the failure to recover from recent wars and a former inept presidency, South Korea's economic power declined rapidly and political instability risked further division of the nation. Korea was vulnerable to international attacks and was highly dependent on foreign aid, such as the United States. However, in 1961, Park Chung Hee, a military general, led a coup that would ultimately revolutionize Korea's economic position. Unlike former Korean presidents, Park believed in the necessity of authoritarianism to maximize economic recovery and his strategy was definite. He took full advantage of his power to silence internal political opposition and to reestablish external relationships; only through stability could Park move forward. Once he confirmed his actions would remain undisturbed, he forced private industries to cooperate with the Park regime and established public economic plans to maintain national growth. Even though Park's authoritarian ideologies and actions spark controversy, he undeniably reinvigorated Korea's economic power and set forth a path that allowed the nation to become the economic tiger it is today.

Keywords: Park Chung Hee, South Korea, Economy, Authoritarianism

1. Introduction

The year is 1953 and the Korean War finally ended, disrupting society and destroying half of the nation's industrial capacity (Eckert, et al., 1991). Beyond the horrors of war, protests and violence further broke Korea's stability under Syngman Rhee's presidency (1948-1960). The instability hindered Rhee's ability to fix the economic problems that further hindered Korea. Unable to maintain itself, the nation required foreign aid, primarily from the United States, and Gross Domestic Product (GDP) per capita (economic output divided by population) was lower than $100 during the 1950s (Tudor, 2012). During this time of economic decline, the military was the only organization in Korea that remained strong (Park, 2008). The survival of the military served well for Park Chung Hee as his power came from the strength of his army. Following his coup in 1961, Park dominated the government using authoritarian practices to revitalize Korea's economy. He first maintained internal and external stability and silenced opposition, such as democracy and work unions, and interacting with foreign nations. Authoritarianism was also used to control the output and power of the private sector, including chaebols (Korean conglomerates) and business owners, by ultimately forcing them to help the government.

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Finally, Park's economic programs redirected manufacturing industries to maximize production and promoted development under full government control. As a result of his authoritarian policies, economic growth is evident. In contrast to 1953, GDP per capita increased to between $120 to $1040 in the late 1970s. Park's strong rule and belief in an authoritarian government marked a major turning point for South Korea and promoted mass economic growth and industrialization. Supported by his military government and bureaucracy, Park Chung Hee was able to control his regime through authoritarian practices and turn South Korea into an economic tiger by restricting freedoms and opposition while promoting stability and significant industrial growth.

2. Establishment of Internal and External Stability

Although Park's authoritarian policies were successful in economic growth, he first ensured internal stability by suspending all political opposition to implement these policies and Park's authoritarian ideology was the drive towards a democratic nation. To achieve a social acceptance of authoritarian practices, Park manipulated the population by criticizing democracy and stated that there were other significant problems to fix, including starvation and poverty, and that "undemocratic emergency measures" were required to solve these problems (Park, 2003). Park further maintained internal stability by restricting the freedoms of the Korea population (Tudor, 2012). For example, protests and labor unions against Park's rule were shut down. These authoritarian restrictions were essential because stability was required to industrialize, otherwise the working population would be unwilling to participate, hindering growth. When the idea of authoritarianism became more accepted by the population, Park could proceed to managing political powers. To avoid political opposition against the authoritarian rule, 4369 politicians were banned from participating in political debate unless they were allowed by the military in 1962 (Haggard et al., 1991). This made it obvious that politicians were not agents of free government, but tools to promote and rubber stamp Park's new policies with some disguise of popular support. The military was also crucial for Park's authoritarian power because that was where his power originated from. To maintain internal stability, the military government suspended political movements and activities so that Park could implement development changes without conflict (Asia for Educators, n.d.). Additionally, Park reinforced the limitation of political opposition by appointing his cabinet members to and limit other political activity through constitutional reform (Park, 2008). By having members of the military a part of the government, a strong sense of loyalty towards Park grew, securing his power. These managed political powers were necessary since unstable politics would cause constant shifts in power and disrupt linear economic growth. Park was able to centralize political power under his authoritarian government and secure constant economic growth. In order to pass any economic reforms, it was essential for Park to maintain internal stability by restricting social and political opposition and making use of a military government under authoritarian rule; only with these conditions could Park keep his power and further his economic plans and expand on foreign interactions.

In addition to ensuring internal stability, Park secured external stability by creating a strong relationship with North Korea to avoid future war and gave South Korea time to develop its economy still under authoritarian rule. War had crippled South Korea's economy. In 1956, the GDP at factor cost (combined net value of varying industries and economic activities) was 145.64 billions won or roughly 188 million USD (The Bank of Korea, 1976). In addition, total exports depleted, reaching only 24.2 million USD and going down to 16.5 in 1958 (The Bank of Korea, 1976). The Republic of Korea army (ROK) had avoided interactions with the North in fear of causing another war (Kamiya, 1980). Instead of avoiding North Korea, like the army proposed, Park believed that the most efficient way to prevent conflict was to directly interact with the North to create a peaceful agreement which would allow South Korea to grow. Park was confident in creating peace between the two Koreas even before the statement overpowering the ROK, which was still fearful of the North. In 1971, the Red Cross from

North and South Korea discussed reuniting families, and in 1972, the South-North Joint Communique was confirmed. The next year, Park announced the Special Statement Regarding Foreign Policy for Peace and Unification in 1973. This policy encouraged North Korea to agree with South Korea that political and military problems needed to be solved (Lyou, 1986). Each of these steps was more progress in reunifying Korea. Park's impact on unification made a lasting and meaningful peace, allowing for South Korea to focus on economic growth and expand foreign relationships without fear of another war. By establishing peace with the North, South Korea could establish foreign interactions and benefit through trade. These foreign relations allowed for more exports of Korean goods, primarily electronics such as black and white televisions. In contrast to the low numbers in the 1950s, total exports grew consistently every year since 1960, near Parks' rise to power, and reached 5,081 million USD in 1975 (The Bank of Korea, 1976). Increased import has proved economic growth; the GDP at factor cost had grown to 8,298.78 (billions won) in contrast to 145.64 in 1954 (The Bank of Korea, 1976). Through Park's policies and social reforms, South Korea was able to establish peace with North Korea, allowing the economy to grow exponentially and prevent any future war without risking undoing all of Park's work.

While Park was making his internal and external changes, the gradual US withdrawal from Asia (1970~75) created fear in South Korean society and that gave Park an opportunity which he capitalized on to increase the power of his regime and its ability to pass reforms and reorganize the economy, promoting growth. Before the US withdrawal, foreign aid was helping South Korea recover its economy. However, Park believed that the aid unbalanced the industries, lowering the impact of heavy industries as light, or secondary, industries "seemed excessively swollen (Asia for Educators, n.d.)." In 1972, light industries had a 60.3% share in total manufacturing output while heavy industries only took up 39.7%. The average annual earnings in Korean Manufacturing was roughly 216 thousand won the same year (Amsden, 1989). Additionally, the total industrial production indexes (production output from manufactured goods) were 43.1 in 1971 and 49.4 in 1972 (The Bank of Korea, 1980). Since the early 1970s, the US was withdrawing soldiers and foreign aid was slowly fading away, leaving Korea in fear of attack and economic downfall (Im, 2006). Park was able to redirect this fear into an acceptance of his authoritarian power. With societal acceptance, not only did Park secure political stability by weakening the appeal of democracy, he was also able to maintain power through the Yushin Coup which he executed in 1972 (Kamiya, 1980). Using his authoritarian practices during the coup, Park created the Yushin Constitution which granted him unlimited presidential terms, making him president for life. During the new Yushin Regime, Park's primary economic reform was to rebalance the impact of heavy industries after having been disrupted by US aid and to mobilize Korea, allowing for national safety and more internal trade. Ultimately, Korea had turned into a garrison state, meaning that the nation was controlled by military power. Park's reason to mobilize South Korea was to bring a promise to the people; that he extended his presidency to continue using authoritarian powers to support heavy industries in order to protect the people. Mass output of heavy industries would greater military preparedness and national defense, promoting safety and internal economic trade. Park was able to build a strong relationship between the political, social, and economic factors of South Korea. By 1977, Heavy and chemical industries output boosted to 50.7%. With large-scale production, more goods are available to trade internally and externally. This boost in heavy industries also allowed for larger annual earnings. The average annual earnings constantly increased each year, the average manufacturing earnings in 1977 was 759 thousand won (Amsden, 1989). With a mobilized nation promoting safety, more internal trade was encouraged. The Wholesale and Retail Trade Index (which represents prices of goods of internal trade) was 49.4 in 1972 and increased to 155.6 five years later (The Bank of Korea, 1980). As a result of heavy industry balancing and extended internal trade through mobilization, the total industrial production index drastically increased to 155.6 in 1977 (The Bank of Korea, 1980). Though the Korean economy was unbalanced by foreign aid,

Park's Yushin Coup was able to prevent any irreversible harm and revitalized the economy by balancing the outputs of heavy and light industries and increasing military capabilities, thus promoting internal trade and national security.

3. Control over Private Economy

Once secured internal and external stability was secured, Park believed it was essential to use the Korean Chaebol to support economic development; however, he needed to control the Chaebol first by limiting their economic freedoms and forcibly redirecting private investment to increase savings. The Chaebol were large industrial conglomerates that were run by a South Korean family. Some Chaebol that still exist today include Hyundai, LG, and Samsung. In 1961 Park passed the Law of Dealing with Illicit Wealth Accumulation, which dictated the choices of private business owners, who Park labeled as profiteers (Amsden, 1989). Park threatened the Chaebol owners by giving them two options: support the government or to go jail and risk the survival of their business. For the Chaebols that agreed to help Park's government, they were fined in which the money was directed to support government projects and savings (Tudor, 2012). Forcing these entrepreneurs to cooperate in government economic reforms and redirected money allowed the economy to grow. From 1963 to 1965, both the government and public and private corporations' savings either increased or remained stable. In 1963, government savings as percent of GNP was 4.41% and grew to 5.83% two years later. Public and private corporation savings as percent of GNP remained stable throughout: the percent was 7.76% in 1963 and was 8.07% in 1965. Government savings also determined how much money could be invested into other industries which Park deemed important. In 1963, government investment into corporations exceeded savings by 2.02 billion won. However, in 1965, government savings was higher than investment by 20.34 billion won (Amsden, 1989). This balance between savings and investment was effective in growing the GNP (total value of produced goods). From 1963 to 1965, the GNP increased from 488.54 to 805.32 billion won (The Bank of Korea, 1976). By forcing private business owners to cooperate with the government, Park was able to increase government savings and investment and was given the opportunity to utilize the wealth to strengthen his regime and country.

Once the government had received enough from the Chaebol to implement economic development plans, Park used his authority to distribute economic resources to increase the industrial outputs of the Chaebols; this greater production would lead to more economic development. Samsung was one of the top Chaebol that Park worked with. In 1965, Samsung total sales were around 26 billion won primarily through food and textile production. However, Samsung's total sales were nearly 25 times bigger in 1975, reaching 640 billion won (Yu and Yan, 2014). This time, sales were equally distributed between food, textiles, and technology such as color television. Many of these products were exported. Another major Chaebol was Hyundai. Total Hyundai sales reached 12.9 billion won in 1968. In 1977, sales reached 147.99 billion won (Chang and Lee, 2009). Similar to Samsung, Hyundai also focused production on manufacturing and heavy industries and exports. In 1965, the total export value was $175.1 million. In 1975, that value became $5,801 million. The Chaebol were not limited to Hyundai and Samsung, many others existed such as LG, SK Corp, Hanjin, and Lotte. The effectiveness of mass production and exports of these many Chaebol are evident through the rise of the GDP value (monetary value of all goods created in a country). From 1965 to 1975, the GDP grew from 750.77 to 9004.14 billion won (The Bank of Korea, 1980). When Park aided the Chaebol, the focus on mass production and exportation of those produced goods allowed the economy to grow significantly.

Not only did Park promote economic growth and authoritarian rule by forcing the Chaebol to cooperate, but also by ordering these companies to maximize exports. Park understood that trade would significantly support South Korea and turn it into a wealthy country (Tudor, 2012). Countries that have stable trade are often stable and willing to support each other, like the USA; this is what Park wanted to achieve and felt that the best way was through rigorous production with minimal opposition from

companies. In 1961, produced exports only took up 26% of all exports. Through industrialization and more production, the percent increased to 84% in 1971 (Chung, 1974). Prior to Park's rule, the total export of Korean goods was $32.8 million. When he implemented authoritarian policies on manufacturing industries, total exports rose to $175.1 million in 1965 (The Bank of Korea, 1980). The increase in produced export percent and total exports proved that South Korea was starting to develop and strengthen the manufacturing and trading sector of the economy. In 1962, GNP growth rate was 2.2% as export growth rates were 31.7%. In 1968, those values grew to 11.3% GNP growth rate and 45.1% export growth rate (Amsden, 1989). The correlation between GNP and export growth supported a strong relationship where more exports meant a higher total value of produced goods. Park understood that external trade would boost manufacturing and create a stronger movement of money throughout Korea's economy and with foreign nations. By maximizing production and export within industries, the economy was allowed to modernize and globalize under the authoritarian policies implemented by Park's government.

4. Utilization of Work Plans

The implementation of Park's five-year economic plans maximized economic growth by forcing various industries to focus on the mass production of specific goods. Park's primary objective was to establish a self-supporting economy and eventually expand to an international level. The first five year plan (1962-1966) was focused on leading companies to mass produce building material such as chemicals and cement in order to strengthen infrastructure (Tudor, 2012). The second plan (1967-1971), similar to the first plan, forced mass production of infrastructural material as well as focus on foreign trade. In 1966, the average capital - labor ratio for cement was 27.72 and continued to grow the following years, reaching 32.06 during 1967-1971. This increase meant that work productivity was improving as well as the value of the product. In order to maximize production, more workers were required. From 1965 to 1969, employment in the manufacturing sector increased by 50% (Amsden, 1989). According to the second economic plan, there was an increased emphasis on trade. In 1971, 84% of all exports were based on goods produced in Korea industries (Chung, 1974). The beneficial impact of increased workers and production is evident through the increased GNP, meaning that the value of products and services have gone up. During the time span of the first five-year plan, the GNP increased from 348.89 to 1,032.41 billion won. The GNP during the second plan also continued to grow, starting with a value of 1,269.95 in 1967 and reaching up to 3,151.55 billion won in 1917 (The Bank of Korea, 1976). Under the control of Park and his five year plans, industries were able to benefit the economy by prioritizing production, causing employment rates and GNP value to increase.

These five-year plans proved successful; thus, Park devised a new program named the Saemaul Undong which focused on structural development in rural areas, allowing villages to be prosperous while still being managed by the government. The Saemaul Undong, or the New Community Movement, worked by giving each village a number of building supplies and the villages that performed the best were given more supplies to further urbanize the rural areas. The overall goal of this program was to promote rural development, eventually helping with economic growth (Ahn, and Boyer, 1984). Even though residents were ultimately restricted to only one type of labor, the program created a balanced system so that hard work resulted in better rewards. Park's development program worked to maximize participation in rebuilding villages (Ahn and Boyer, 1984). From 1971 to 1979, the number of participating villages increased from 33,267 to 36,271. During the same years, the number of individual participants grew from 7,200 thousand to 242,078 thousand (Lee, 1990). With a larger workforce comes faster structural and economic development. In 1975, the gross value added from construction was 316,651 won and reached 1,616,048 won in 1978 (The Bank of Korea, 1980). By directing people into working not only for rural development but also for national economic prosperity, the Saemaul Undong program was able to promote economic development through mass construction.

5. Conclusion

Using his authoritarian policies and power of the military government, Park Chung Hee was successfully able to develop a strong South Korean economy. He first stabilized by using his authoritarian policies to silence any political opposition and prevent any further conflicts that would hinder economic growth and modernization. Through managing internal and external stability, the government was given the opportunity to implement changes into Korea's economy and workforce. Park forced changes in the private sector and released government-led plans that aimed to help economic growth. The changes that Park brought were effective in rebuilding Korea's economy. Prior to his rule, conflicts through war and a broken rule under president Rhee harmed Korea's ability to grow. Not only was Park able to fix the problems that destroyed Korea before, he was able to stabilize Korea so that future generations could also focus on economic growth rather than having to reorganize the nation. His legacy in the development of Korea remains significant to this day. Ultimately, through authoritarian powers, Park Chung Hee was able to redirect society in his favor in order to ensure maximal economic growth and turn Korea into a modern economic tiger.

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