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Title: Stress Induction via the Oculus Rift and Its Effects on Probabilistic Reasoning

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Stress Induction via the Oculus Rift and Its Effects on Probabilistic Reasoning

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Candidate for the degree

Bachelor of Sciences

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Departmental Distinction in Biochemistry/Biology

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STRESS INDUCTION VIA THE OCULUS RIFT AND ITS EFFECTS ON PROBABILISTIC REASONING

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Abstract

In order to determine if stress affects Probabilistic Reasoning, we observed the relationship between cortisol levels and galvanic skin response in relation to a task involving context reliance in decision making. The data of 32 subjects were analyzed. Stress induction was performed via oculus rift while Probabilistic Reasoning was measures via the jumping to conclusion task. The hypothesis that the oculus rift can be a successful inducer of stress was supported only with the galvanic skin response technology. The levels of cortisol and alpha-amylase of the subjects did not support the hypothesis. The second hypothesis stating that the stress induction via the oculus rift will decrease Probabilistic Reasoning measures was not supported. This study shows that the new technology of the oculus rift can be used to induce stress.

Background Information

This research was done on the stress inducing effects of the Oculus Rift and how that stress induction affected Probabilistic Reasoning (Bayesian Processing), or the idea that an optimal combination of sensory evidence and prior knowledge is required to interpret stimuli in present context. Bayesian Processing plays a significant role in the reward system of the brain and therefore aides in the repetition of actions. It can be broken down into 3 categories that ultimately aide in making a decision: the *prior*, *likelihood*, and *posterior* (Notredame et.al 2014). The *prior* is the probability of summarizing prior knowledge before receiving any sensory information and is useful in the top-down processing of the brain. This type of processing is significant in conceptual formation and abstract thinking. The *likelihood* is the probability provided by sensory organs and supporting evidence such as contextual clues. This plays a critical role in bottom-up processing, which is used for the identification of sensory evidence. The *posterior* is the probability of the percept resulting from the combining of the *prior* and the *likelihood.* In the present study, Bayesian processing was studied through one task, the Jumping to Conclusion task.

Probabilistic Reasoning, in general, is a hierarchal inference process involving top-down and bottom-up processing, which is important to research and learn more

information about due to its connection to cognitive and perceptive aspects of schizophrenia and other psychological disorder (Notredame et.al 2014). This will provide a better understanding for cognition and perception and how they interact when contributing to consciousness. This combination of perception and cognition is working together at all times when in terms of functioning (MacDonald et.al 2009). Conceptually, if this combination becomes abnormal, psychological disorder may occur. For example, schizophrenia, a severe disorder characterized by hallucinations and delusions, may be a consequence of aberrant processing in the reward system of the brain (Fletcher 2010). Dopamine levels are deregulated within the brains of many individuals with schizophrenia, which could contribute to delusions and hallucinations, phenomenon that could also be related to altered top-down and bottom-up processing (Keefe et.al 2011). Delusions, for example, can be conceptualized as the outcome of an irregular top-down processing whereas hallucinations are the effect of the bottom-up processing being irregular (Fletcher 2010).

While Probabilistic Reasoning can be an abstract concept, certain neurotransmitters have been implicated in its function due to the involved signaling and networking amongst different regions of the brain. One specific example of this is in the human brain's reward system, which plays a significant role regulating consistency of actions or thoughts (if an action is beneficial for an organism's survival, this system helps encourage that the repetition of that action) (Deserno et.al 2013). The basal ganglia portion of the brain in the main component of the loop that drives the reward system. The mesolimbic dopamine pathway is a group of neurons that that connect the ventral tegmental area to the nucleus accumbens through the cortico-basal-ganglia-thalamic loop (Yager et.al 2015). The cortico-basal ganglia-thalamic loop includes several regions of the brain which are the cerebral cortex, basal ganglia, and thalamus. The cerebral cortex is involved in higher level thinking. The basal ganglia has many functions, but of import to this study is its involvement and interaction with dopamine signaling. The thalamus is known as the command center of the brain which is where the messages are relayed throughout the rest of the brain and body. Therefore, eliciting a direct pathway to guiding the behaviors of an individual (Yager et.al 2015).

Dopamine is one of the neurotransmitters that is significantly involved in this system and is released whenever a beneficial action or thought is completed, which causes an update within the prior to ensure the repetition of beneficial tasks. Dopamine levels are increased in the basal ganglia and decreased in the prefrontal cortex in those diagnosed with schizophrenia. In some cases, psychotic disorders may arise due to an aberrant assignment of novelty to associations (Kapur et.al 2005), and therefore, this deregulation may be integral to the development of schizophrenia. Consistent with this, individuals diagnosed with schizophrenia have shown behavioral impairments in reinforcement learning (Deserno et.al 2013). Collectively, this research illustrates the significance of dopamine regulation and its correlation to schizophrenia.

Glutamate is another neurotransmitter that is involved with Probabilistic Reasoning processes. Glutamate has been shown to affect the amount of dopamine released via a cascade effect initially induced by stress. This then deregulates the levels of dopamine throughout the brain, and, by extension, can affect the Probabilistic Reasoning of an individual (Hoskin 2014). Deregulation of the glutamatergic system occurs in the prefrontal cortex, hippocampus, and amygdala due to stress; suggesting that glutamate is significant in cognitive alterations and neuropsychiatric disorders. (Graybeal et.al 2012). The deregulation of glutamate has been linked to deficits in learning and memory functions such as cognitive flexibility and working memory, both of which are symptoms to schizophrenia.

Since stress is a key deregulator of both glutamate and dopamine, cortisol, a common glucocorticoid, is a common biomarker for stress within the body (Corcoran et al. 2003). Cortisol responds rapidly to a wide range of environmental and internal demands, referred to as stress (Kirschbaum, Hellhammer 2000). Cortisol is synthesized and released into the circulating blood from the adrenal glands. It is represented by two types known as bound and unbound or free cortisol. Both are present within the blood; however, free cortisol can move without resistance throughout the body due to not being bounded to proteins within the blood. Cortisol also yields a four carbon fused ring steroid structure, thus giving cortisol an overall nonpolar structure. A nonpolar steroid is hydrophobic; therefore, allows the free cortisol to travel across cellular membranes. Free cortisol is present within the saliva and represents about 2-15% of the total cortisol

throughout the body. Although the percentages are low, free cortisol is still a valid index of stress levels within the body. Bound cortisol binds to large proteins such as human serum albumin (blood albumin) and cortical-binding globulin (transcortin) in the blood (Corcoran et al. 2003). Cortical-binding globin contains a hydrophobic pocket within its protein structure where cortisol interacts. The nonpolar, hydrophobic pocket interacts with the oxygen atoms on cortisol allowing for a strong binding interaction. Human serum albumin has a greater binding affinity for small, negatively charged hydrophobic molecules (Dockal et al. 1999). Cortisol is well suited for this binding due to its numerous oxygen molecules and being a hydrophobic compound. Human serum albumin contains two specific binding sites that favor small heterocyclic compounds. This is the location of where cortisol interacts with the protein. The cortisol that is synthesized and released throughout the body from the adrenal glands affects those neurotransmitters and thus, affecting the probabilistic reasoning of an individual.

Alpha-amylase is another protein structure that is increased by acute stress induction, therefore making this protein a non-invasive marker for detecting sympathetic nervous system activity (Rohleder et al. 2004). Alpha-amylase is mainly involved with the digestion and breakdown of starch and glycogen inside the oral cavity (Engert et al. 2011). This protein is synthesized and released by acinar cells, which are responsible for 80% of major salivary glands (Rohleder et al. 2006). Both cortisol and alpha-amylase increase due to stress induction; however, alpha-amylase levels have shown to increase quicker and to a larger extent when compared to cortisol. Although both are reliable biomarkers for stress, this suggests that alpha-amylase is more reliable (Takai et al. 2004). Similar to cortisol, alpha-amylase levels can be an indicator of biological measures of probabilistic reasoning by representing stress within an individual. The relationship between stress and alpha-amylase is very important in understanding its effects against probabilistic reasoning. Cortisol and alpha-amylase both exhibit a lagging effect thus showing each of them to have similar kinetic profiles. Their release rate can determine how quickly the individual being stressed will begin to show decreases in probabilistic reasoning measures (Engert et.al 2011).

It is evident that extreme variations in Probabilistic Reasoning can be implicated in disorders such as schizophrenia. Less is known about how it varies in non-clinical

populations, however. Stress has been shown to promote deficits in the reward system (Berghorst et.al 2013). This stress response is involved with dopamine, glutamate, and Probabilistic Reasoning through the hypothalamus-pituitary-adrenal axis (HPA axis), commonly, referred to as the stress cascade due to the sequential events that occur (Corcoran et.al 2003). The stress cascade has a simple mechanism similar to a cascade effect. The release of cortisol stimulates two regions of the brain known as the hypothalamus and pituitary. Cortisol is released from the adrenal glands due to adrenocorticotropic hormone (ACTH) and corticotropin-releasing hormone (CRH). ACTH is released from the anterior lobe of the pituitary gland. CRH is released from the hypothalamus (Corcoran et.al 2003).The effect of stress on the hypothalamus leads to cognitive deficits, such as poor memory and impaired feedback. Both of which are symptoms of those diagnosed with schizophrenia. Impaired feedback, within the reward system, is due to the deregulated levels of dopamine and glutamate (Baudonnat et.al, 2013). Through this stress cascade, dopaminergic and glutamatergic pathways are altered, causing the deregulated levels of each throughout the brain. Forty to seventy-five percent of dopaminergic neurons have glucocorticoid receptors on them (Corcoran et.al 2003), thus showing an interaction between cortisol and parts of the brain significant for probabilistic reasoning. This shines light on the theory that schizophrenic symptoms may arise within those who are not diagnosed with a cognitive disorder but may be exhibiting increased cortisol levels or stress.

In this study, the Oculus Rift was used to induce stress. It involved a virtual reality technological device, or ocular lens, which is placed over the eyes to simulate a three dimensional environment. There were two conditions that included an experimental and control group. The experimental condition was a 3-minute scary scenario, designed to induce fear, based on the video game *Slender*. The control condition involved subjects wearing the Oculus Rift but seeing a camera image of what would be in front of them in the natural environment.

The purpose of this study was to two-fold: 1) to observe if the experimental Oculus Rift condition was sufficient to induce stress in participants; and 2) to observe if this stress induction affected individuals' Probabilistic Reasoning (assessed with a bead counting task). We hypothesized that the Oculus Rift will induce stress amongst the

participants and this stress induction will cause a deficit in Probabilistic Reasoning measures.

Methods

Participants

The 36 individuals were gathered from the Albright College Community. There were 15 males and 21 females, between the ages of 18-22 years old, who participated. Participants were excluded from the study if they had consumed food an hour before testing due to the possible spike in cortisol from such an event. Participants were not permitted to ingest any alcohol 12 hours before testing or neuroactive drugs a week before testing. The ingestion of these cause the brain to respond differently than the normal person. Alcohol is a depressant and can therefore, inhibit neuronal response. Whereas, neuroactive drugs, such as caffeine, can excite neuronal response. Both affect the normal neural pathways and cortisol production of an individual.

Oculus Rift

The oculus rift, purchased from Oculus, is a virtual reality technology device which is placed over the eyes to simulate three dimensional scenarios. The Development Kit 2 was the model used for experimenting. The experimental condition used a 3 minute clip that elicited a scary scenario. This was used to induce stress upon the test subjects. The control condition used a neutral scenario which simulated normal vision in real time. This condition matched the length of the experimental to minimize deviation. The rift was manufactured by Oculus VR. The display resolution was 960 x 1080 per eye, OLED technology. It also included gyroscopic, accelerometric, and magnetometric sensors. The oculus rift contained a 1000Hz update rate as well as a 360° view.

Probabilistic Reasoning task

The jumping to conclusion task is a method used to determine Probabilistic Reasoning being used by participants. The Jumping to Conclusions task involved two jars of beads in which there were two conditions, an easy task and a hard task. The easy condition contained two jars with an 85:15 ratio of black to yellow and yellow to black beads, respectively. The hard condition contained two jars with a 60:40 ratio of black to yellow and yellow to black beads, respectively. The participants were told the experimenter was picking a random bead from one of the jars and their goal was to accurately pick the jar, although they were not allowed to see it. The experimenter would pick one bead at a time, and participants were allowed one guess, but could choose to observe as many beads as they wanted before making their decision. While participants were told the selection was random, a script was used to the same bead order was chosen for each participant. The metrics were the amount of beads that were drawn before the participant came to a decision, and whether or not participants guessed before the 3rd bead selection, and indication of a jump to a conclusion.

Galvanic Skin Response

The galvanic skin response (GSR) technology is a method of exosomatic recording of electrodermal activity by the applying external current to the skin. The GSR technology involved attaching two electrodes to the distal phalanx of the test subject's index and ring fingers. The current created recorded skin conductance in µSiemens. The distal portion of the index finger and the ring finger were wetted with a wet paper towel to ensure electrical conductivity of the skin. The skin conductance was recorded to validate the experimental condition induced stress. The galvanic skin response technology measures the sweat that is produced by the miniscule pores on the fingertips of an individual. The greater the change in skin conductance, the greater the effect of stress induction. The iWorx214 was the unit used for data acquisition. There were 10 marks recorded throughout the clip. They are as follows: placing of the oculus rift on the test subject; the start of the scenario; 60sec into clip; 120sec into clip; 180sec into clip; 188sec into clip (monster shows) ; 198sec into clip (10sec after monster is

shown); during bead task; and post-test saliva sample. The LabScribe software was used to analyze the data recordings from the electrodes. The parameters set were as followed: 200 samples/sec; DIN8 mode; 30sec display time.

Pulse Oximeter

The pulse oximeter is a non-invasive method used to record the percentage of oxygen within the blood, heart rate in beats per minute (bpm), and the pulse in volts. It uses two light-emitting diodes (LEDs) to record these measurements. One LED is red with a wavelength of 660nm and the other LED is infrared with a wavelength of 940nm. The pulse oximeter probe was attached to the thumb of the test subjects. This is the standard finger for use when the index finger is occupied. The measure of oxygen percentage was used for the determination of blood oxygen levels to validate the experimental condition induced stress. The heart rate and pulse were recorded by the same LED diodes. The pulse oximeter also used the iWorx214 unit and the LabScribe software for data acquisition and analysis.

Procedure

The test subjects were asked to passive drool into the test tube before any of the testing had occurred. The ring and index finger were dampened with a moist paper towel to ensure conductivity. The galvanic skin response electrodes were then placed on the ring and index finger of the test subject. The pulse oximeter was placed on the thumb. The oculus rift was then applied over the eyes of the test subject. Either the control or experimental scenario were shown based on a randomized, counterbalanced protocol for each participant. Then additional measures were recorded to determine the changes in galvanic skin response and heart rate. These additional changes were as followed: Greatest overall experiment change; Change from monster to 10s after monster; Change from oculus rift on to 10sec after; Greatest change with the rift on; Overall change 40sec before & 40sec after (-40/+40) the monster point. Then the test subjects were given a Probabilistic Reasoning task known as the jumping to conclusion task.

After this task, another saliva sample was collected by passive drool. Both saliva samples were placed on ice immediately to minimize protein decay.

Enzyme-Linked Immunosorbent Assay (Cortisol)

Antibodies and Reagents: A cortisol enzyme-linked immunosorbent assay kit from Salimetrics was used to quantify the cortisol samples. The kit was stored at 2-8°C. A 96-well plate coated with monoclonal anti-cortisol antibodies was used to hold and bind the cortisol. The kit came with standard solutions including concentrations of 3.0µg/dL, 1.0µg/dL, 0.333µg/dL, 0.111µg/dL, 0.037µg/dL, and 0.012µg/dL. Control samples were also given in the kit. These were deemed high concentration and low concentration controls. A wash buffer was also given in the kit for washing of the wells when admitted. Tetramethylbenzidine was the substrate used for the enzymatic reaction to occur. A 3M sulfuric acid was used as stop solution to stop the enzymatic reaction.

Sample Preparation: Saliva samples were collected by passive drool before and after testing. The cortisol samples were placed in a -20°C freezer for storage until the time of the ELISA. Reagents were prepared first by bringing them all to room temperature (25°C). It is important that the plate strips are brought to room temperature before the removal of the tinfoil, as the humidity can affect the antibodies on the plate. The 1X wash buffer was prepared with 12mL of wash buffer and 108mL of deionized water. This provided the necessary amount of 120mL the washing steps.

Procedure: A plate layout was determined in order for the assignment of the saliva samples to the different wells. 24mL of the assay diluent was pipetted into a disposable pipette tube. 25µL of the samples were pipetted into the assigned wells based off of the plate layout determined earlier. This included standards, controls, and saliva samples. Only assay diluent was pipetted for the blanks. The enzyme conjugate was diluted 1:1600 by adding 15µL to the 24mL tube of assay diluent. This tube was centrifuged to ensure the diluted solution was mixed appropriately. 200µL of the 1:1600 solution was pipetted into each of the wells using a multichannel pipette. The plate was then mixed for 5 minutes at 500rpm and incubate at room temperature for 60 minutes. The plates were then washed 4 times with the 1X buffer. Washing was done by gently squirting the

wash buffer into each well with a multichannel pipette. The plate should be blotted on paper towels before turning upright. 200µL of the tetramethylbenzidine substrate solution was added to each well. Then the plate was mixed for 5 minutes at 500rpm in the dark (covered) for 25 minutes. 50µL of 3M stop solution was added to each well with a multichannel pipette. After this, the plate was mixed for a final time for 3 minutes at 500rpm. Continued mixing until the green color disperses and only yellow was seen. The plate was read at 450nm to recorded absorbance values.

Enzyme-linked Immunosorbent Assay (Alpha-Amylase)

Antibodies and reagents: An alpha-amylase enzyme-linked immunosorbent assay kit from IBL International was used to quantify the alpha-amylase within the saliva samples. Standard sample concentrations were Standard 1 (400µg/mL); Standard 2 (200µg/mL); Standard 3 (100µg/mL); Standard 4 (50µg/mL); Standard 5 (0µg/mL). Standard 1 was prepared with 10µL of reconstituted stock standard and 3mL of diluted sample buffer. Standard 2 was prepared with 100µL of Standard 1 and 100µL of diluted sample buffer. Standard 3 was prepared with 100µL of Standard 2 and 100µL of diluted sample buffer. Standard 4 was prepared with 100µL of Standard 3 and 100µL of diluted sample buffer. Standard 5 was prepared with 200µL of diluted sample buffer. The substrate solution was provided within the kit as well as all other necessary stock solutions and controls.

Sample Preparation: Saliva samples were collected by passive drool before and after testing. The cortisol samples were placed in a -20°C freezer for storage until the time of the ELISA. Reagents were prepared first by bringing them all to room temperature (25°C). It is important that the plate strips are brought to room temperature before the removal of the tinfoil, as the humidity can affect the antibodies on the plate. The diluted sample buffer was prepared with 10mL of sample buffer and 90mL of deionized water. This provided the necessary amount of 100mL of diluted sample buffer. The samples were prepared with 10µL of saliva sample and 3mL of diluted sample buffer. The control samples were prepared with 10µL of control (reconstituted) reagent and 3mL of diluted sample buffer.

Procedure: Pipetted 10µL of each prediluted standard, control, and sample into the respective wells of the microtiter plate. Pipetted 200µL of the substrate solution into each will. Then the plate was shaken carefully within the ELISA instrument. Incubated the plate at room temperature for 3 minutes. Measurements of optical density were recorded at 405nm. Then incubated for another 5 minutes at room temperature. Measurements of the optical density were recorded again at 405nm.

Results

Table 1: The frequency and percentage for each condition

Condition			
		Frequency	Percent
Valid	control	18	50
	manipulation	18	50
	Total	36	100

Table 2: The frequency and percentage for the gender of the participants

Gender			
		Frequency	Percent
Valid	Male	15	41.7
	Female	21	58.3
	Total	36	100

Table 3: The frequency and percentage for the different ages of the participants

Age			
		Frequency	Percent
Valid	18	6	16.7
	19	3	8.3
	20	12	33.3
	21	11	30.6
	22	4	11.1
	Total	36	100

Table 4: The frequency and the percentage for the races of the participants

Race			
		Frequency	Percent
Valid	white	22	61.1
	black	5	13.9
	hispanic	4	11.1
	asian	2	5.6
	mixed race	3	8.3
	Total	36	100

We performed a *T*-test to determine if skin conductance of our manipulation was different from the skin conductance of our control conditions when the oculus rift was first put on the heads of participants, indicating the start of the experiment. The results indicate that the groups were not significantly different, $t(34) = 1.172$, $p = 0.249$. This suggests that the group had similar levels of stress, as measured by the GSR, when they began the study.

We performed a *T*-test to determine if skin conductance of our control was different from the skin conductance of our manipulation condition overall change throughout the whole experience, including the oculus rift being on and off the participants. The results indicate that the groups were not significantly different, $t(34) = -1.231$, $p = 0.227$. This suggests that the overall experience of the testing did affect the physiology of the participants in both conditions and induced stress. We also performed a T-test to determine if the heart rate of our control condition was different from manipulation conditions for the overall experiment. The results indicate that there was a significant difference, $t(34) = -2.104$, $p < 0.05$. This suggests that the two groups had different heart rates.

We performed a T-test to determine if skin conductance of our control was different from the skin conductance of our manipulation condition at 10 seconds after the monster's appearance. The results indicate that the groups were significantly different, $t(34) = -3.938$, $p < 0.01$. This suggests that the appearance of the monster did

affect the physiology of the participants in both conditions, and induced more stress in the manipulation condition. We also performed a T-test to determine if the heart rate of our control condition was different from our manipulation condition at 10 seconds after the monster's appearance or the 180s mark. The results indicate that there was a significant difference, $t(34) = -3.602$, $p < 0.01$. This suggests that there was a difference between the control and manipulation condition at this marker; thus showing the manipulation condition was more stressed.

We performed a T-test to determine if the skin conductance of our control was different from the manipulation conditions at the moment the oculus rift was placed on and 10 seconds after the oculus rift was placed on. The results indicate that the groups were not significantly different, $t(34) = 1.949$, $p = 0.060$. This measure was taken in order to allow for a physiological response. This suggests that the initial physiological change to the application of the oculus rift was not immediate and was longer than 10 seconds. This was the same between both groups. We also performed a T-test to determine if the heart rate of our control condition was different from our manipulation condition at the moment the oculus rift was placed on and 10 seconds after the oculus rift was placed on. The results indicate that there was not a significant difference, $t(34) = 0.78$, $p = 0.540$. This measure was taken in order to allow for a physiological response. This suggest that the initial physiological change in heart rate to the application of the oculus rift the same between each of the groups.

We performed a T-test to determine if the skin conductance of our control condition was different from our manipulation condition for the overall experiment while the oculus rift was on. The results indicate that the groups were significantly different, $t(34) = -5.462$, $p < 0.01$. This measure excluded the bead task marker and the post-experiment saliva sample. This indicates that our peak difference in GSR during the experiment was larger for the manipulation group, suggesting a larger stress response compared to controls. We also performed a T-test to determine if the heart rate of our control condition was different from our manipulation condition for the overall experiment while the oculus rift was on. The results indicate that there was a significant difference, $t(34) = -2.508$, $p < 0.05$. This measure excluded the bead task marker and

the post-experiment saliva sample. This suggests that the groups differed in this comparison.

We performed a T-test to determine if the skin conductance of our manipulation condition 40 seconds before and 40 seconds after the monster had appeared was different from our control condition 40 seconds before and 40 seconds after the 180sec marker during the task performance. The results indicate that there was a significant difference, $t(34) = -8.165$, $p < 0.01$. This suggests that the individuals in the manipulation condition exhibited more stress than the control condition; thus, the electrophysiological evidence indicates the oculus rift did induce stress. Skin conductance increased much greater within the test subjects of the manipulation group than the test subjects of the control group at the same time marker. We also performed a T-test to determine if the heart rate of our manipulation condition 40 seconds before and 40 seconds after the monster had appeared was different from our control condition 40 seconds before and 40 seconds after the 180sec marker during the task performance. The results indicate that there was a significant difference, $t(34) = -2.082$, $p < 0.05$. This suggests that the individuals did exhibit electrophysiological evidence that the oculus rift did induce stress. The heart rate increased much greater within the test subjects of the manipulation group than the test subjects of the control group over the same period of time.

We performed a 2x2 mixed design ANOVA to determine if there was an interaction between the manipulation and the timing of the bio-sample collection. There was a significant change in alpha amylase overall between the control and manipulation conditions, $F(1,34) = 5.527$, $p < 0.05$. This suggest the overall change in alpha-amylase was affected by the induced stress. However, the significant change in alpha-amylase was not specific to the manipulation group, $F(1,34) = 1.902$, $p = 1.77$. Both data suggest that the alpha-amylase was affected in both conditions.

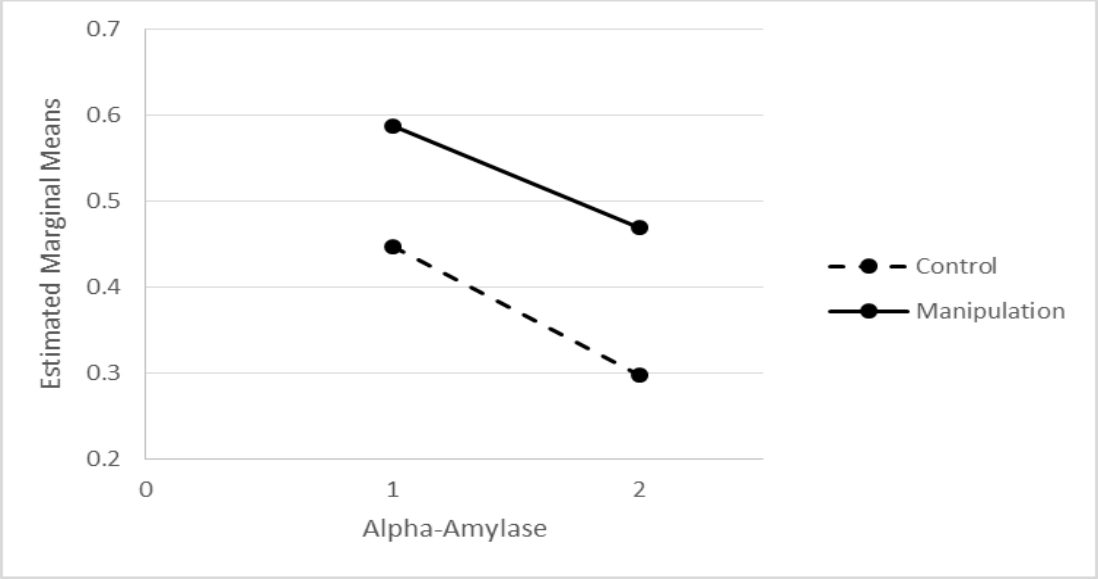

Figure 1: Graphical depiction of the estimated marginal means versus alpha-amylase. The control condition is represented by the dotted-line. The manipulation condition is represented by the solid line. Point 1 on the x-axis is the pre-experiment sample and point 2 is the post-experiment sample.

The graph depicted in Figure 1 shows a decrease in alpha-amylase in both the control and the manipulation conditions. However, the manipulation condition shows higher levels overall than that of the control condition at both time points. This may suggest that the manipulation condition elicited a greater overall alpha-amylase concentration within the test participants' saliva. It was expected that the alpha-amylase would increase in both conditions with the manipulation condition eliciting a greater increase in alpha-amylase than the control.

We performed a T-test to determine if the overall cortisol was different between the control and manipulation conditions. The results show that there was a significant difference, $t(1,32) = 21.613$, $p < 0.01$. This suggest the overall change in cortisol was affected by the induced stress. The significant change in cortisol was also specific to the manipulation group, $t(1,32) = 11.481$, $p < 0.05$. Both data suggest that the cortisol was affected in both conditions. The cortisol levels decreased between the pre-saliva and post-saliva sampling for both of the conditions; however, the manipulation showed a lesser decrease.

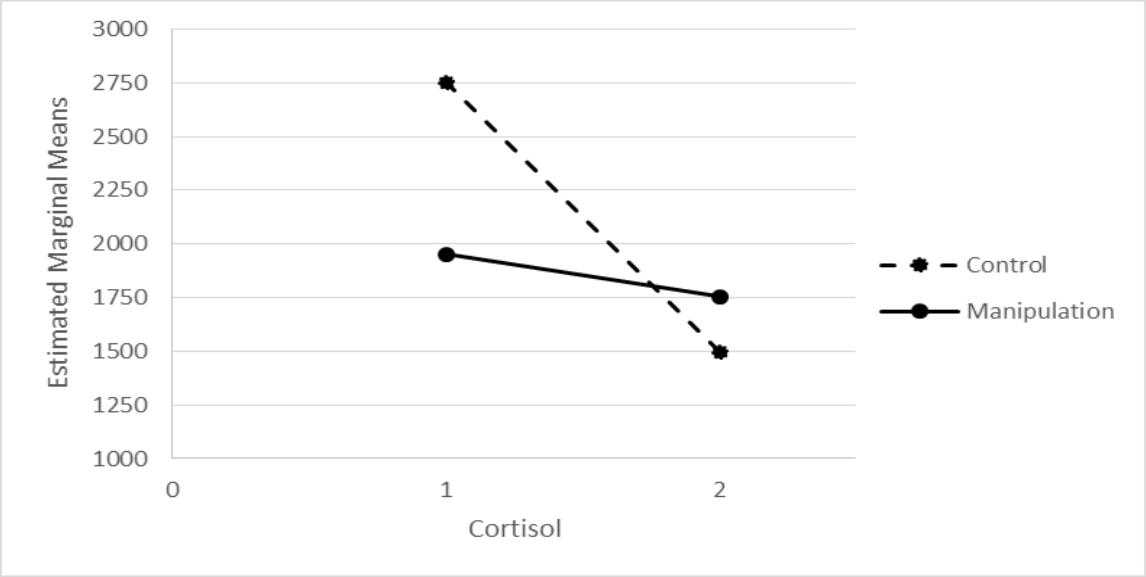

Figure 2: Graphical depiction of the estimated marginal means versus cortisol. The control condition is represented by the dotted-line. The manipulation condition is represented by the solid line. Point 1 on the x-axis is the pre-experiment sample and point 2 is the post-experiment sample.

The graph depicted in Figure 2 shows a decrease in cortisol in both the control and the manipulation conditions. However, the decrease in the manipulation condition is much less than that of the control condition. It was expected that the cortisol would increase in both conditions with the manipulation condition eliciting a greater increase in cortisol than the control.

We performed a T-test to determine if the number of draws for the easy condition of the bead task for the control condition was different from the number of draws for the easy condition of the bead task for the manipulation condition. The results indicate that there was a significant difference, $F(1,34) = 13.081$, $p < 0.01$. This suggest that the control condition required a different number of draws for the easy condition of the bead task than the manipulation condition.

We performed a T-test to determine if the number of draws for the hard condition of the bead task for the control condition was different from the number of draws for the hard condition of the bead task for the manipulation condition. The results indicate that there was not a significant difference, $F(1,34) = 0.742$, $p = 0.395$. This suggest that the control condition did not require a different number of draws for the easy condition of the bead task than the manipulation condition.

A Chi-Square goodness of fit test was performed to determine whether the conditions differed in whether or not participants jumped to conclusions in the hard condition [$\chi 2$ (1, 36) = 1.87, p = 0.171] and in the easy condition [$\chi 2$ (1, 36) = 0.114, p = 0.735]. In neither case did the groups differ in their expected and observed outcomes. This suggests that the measure of probabilistic reasoning amongst the test subjects was not altered by the induced stress of the oculus rift.

Discussion

Our hypothesis was that the oculus rift could be a successful inducer of stress; this was supported only with the galvanic skin response technology. The results show an increase in the electrophysiological measures of skin conductance and heart rate due to the oculus rift manipulation condition. This is shown by the galvanic skin response technology that measures the sweat that is produced by the miniscule pores on the fingertips of an individual. The greater the change in skin conductance, the greater the effect of stress induction (Critchley 2002). The increase in heart rate is shown by the pulse oximeter technology that measured the heart rate directly. Our data therefore suggests that this paradigm can be used as a means of stress induction in future testing. However, the change cortisol and alpha-amylase levels did not support the electrophysiological data.

The levels of cortisol and alpha-amylase of the subjects both decreased from the start to the end of the experiment, which was not expected. Both the cortisol levels and the alpha-amylase levels were expected to increase with stress induction. Although both the control and manipulation conditions of the cortisol decreased, the manipulation decreased to a much lesser degree (Figure 2). It is possible that the samples decreased due to the freeze-thawing of the saliva samples deteriorating the proteins within the saliva between ELISA runs. Although cortisol is a stable protein, freeze-thawing of the saliva countless times can breakdown the protein; therefore, affecting the amount present within the saliva at the time of quantifying (Kang 2010). This raises the

possibility that the manipulation condition had more cortisol produced in the post-saliva sample than the post-saliva sample of the control condition. As the decrease from time 1 to time 2 was significantly smaller in the manipulation condition. Alpha-amylase also decreased in both the control and manipulation conditions (Figure 2). These decreases were also not expected to occur based on the literature (Ernest et.al 2011). It is also possible that this could result from multiple freeze-thawing of the saliva samples (Kang 2010). The freeze-thawing could have caused a deterioration in the protein structure; thus eliciting a smaller recorded measurement. Protein breakdown via freeze-thawing is possible due to the electrophysiological measures supporting a stress induction while the biological measures did not support any stress induction.

The second hypothesis stating that the stress induction via the oculus rift will decrease Probabilistic Reasoning measures was not supported. Probabilistic Reasoning measures showed no significance in both the easy and hard conditions of the bead task between the control and manipulation conditions. There was stress induction, as suggested by the electrophysiological data from the skin conductance and heart rate. However, the stress induction did not affect the test subjects' probabilistic reasoning within the bead task. Stress was expected to decrease the probabilistic reasoning measures within individuals (Berghorst et.al 2013). Our results may owe to the test subjects not experiencing the stress induction as quick. Alpha-amylase and cortisol exhibit positive time lags due to their release of kinetics (Engert et.al 2011). This rate lag may cause the probabilistic reasoning measures to be affected either before or after the bead task. The test subjects may have recovered from the stress induction before the bead task.

The stress and probabilistic reasoning interaction is very important in understanding schizophrenia. Those who are diagnosed with schizophrenia exhibit symptoms that are similar to the effects of stress on a healthy individual (Freeman 2006). Stress is known to disrupt the reward system of individuals. Less is known how this reward systems is affected in non-clinical individuals. Therefore, studying the interaction of stress and probabilistic reasoning within the general population gives a greater insight to the diagnosed individuals. Although this research did not validate this connection, the oculus rift has been shown to induce stress and further research is

necessary for finding out more about the interaction of stress and probabilistic reasoning.

Future research would include another analysis involving the recording of the same biomarkers for stress, alpha-amylase and cortisol. The quantifying of these proteins will be done on the same day to prevent the degradation of the proteins via freeze-thawing. Future research may also include the saliva sampling to the collected at a different time; thus, allowing the alpha-amylase and cortisol levels to be more accurate and less affected by the kinetic lag. The jumping to conclusion task (bead task) will remain in future research unless another option of recording probabilistic reasoning is suggested. This experiment shows that the oculus rift technology can be used as a stress induction mechanism. This can help other studies in the future by providing a simple, harmless, and successful mean of inducing stress for studies that may require stress induction.

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