EXPLORATORY BEHAVIORAL AND NEURAL EFFECTS OF INFLAMMATION-INDUCED SICKNESS BEHAVIOR

By

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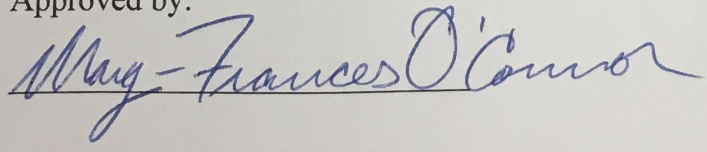

A. Abstract

Sickness behavior entails behavioral changes such as anxiety or fatigue due to increased circulatory proinflammatory cytokine (IL-6) levels. By crossing the blood-brain barrier, IL-6 induces widespread but poorly understood neurological changes that potentially shift the body's energetic prioritization toward immune recovery. The present study focuses on identifying the mechanisms and behavioral consequences of human sickness behavior on exploration. Sickness behavior was hypothesized to demonstrate a decrease in natural human exploration by dopaminergic mechanisms. We assessed this behavioral phenomenon in participants' exploration of a novel virtual open-field test and a gambling task. Upon receiving the vaccine, participants reported decreased attentional impulsivity on the BIS-11 scale and decrease perceived stress. Participants traversed less and paused more in the virtual-reality open-field test when vaccinated, both of which were mediated by immune reactivity as measured by change in IL-6 levels. These findings support goals of sickness behavior: less curiosity to explore virtual-reality is less cognitively expensive, so energy otherwise invested in pursuing curiosities can focus on immune recovery instead. Further studies on how inflammation changes behavior and neurological regions implicated could improve patient quality of life through better diagnosis and management of inflammation-induced side effects such as depression.

B. Background

B.1. Inflammation and Sickness Behavior

The complexity with which the human body interacts with its external environment brings with it a range of challenges for the body to overcome. While the surrounding world provides the nutrients and biological building blocks for survival, it also hosts a number of organisms that can bring about damage or death to the human body. Further, the human body often naturally exhibits genetic mutations or predispositions that can pose an internal threat to itself. The complex system that identifies and neutralizes these threats to the human body is what constitutes the immune system.

This intricate system of cells, spread throughout the body in organs and circulatory pathways, can be separated by two distinct functions: innate and adaptive. The innate immune system facilitates a non-specific attack of anything that is not made by the individual and healthy. For instance, if one scrapes their knee while running in the schoolyard, the immune system notifies the body that the skin has been infiltrated by non-self particles. In this instance, the distinction between dirt, rocks, and specific chemicals is not made. The adaptive immune system, on the other hand, is specific to a particular types of infiltrators (pathogens), and can 'learn' over time to attack its specific pathogen more rapidly. For instance, if the previously mentioned scrape had allowed the influenza virus to pass the skin barrier, the adaptive immune system recruits cells that have a specific ability to bind the flu virus cell, and creates more cells that will recognize the flu virus pathogen the next time that it gets into the body.

When one is sick, the immune system mobilizes by releasing proinflammatory cytokines, which are immune proteins equivalent to Hansel and Gretel's proverbial breadcrumbs; they lead other immune cells, both innate and adaptive, to the region with the most cytokines, allowing for a more mobilized attack on a pathogen and thus, a more rapid health recovery for the individual.

If these proinflammatory cytokines are chronically left unchecked by not returning to one's healthy, baseline immune function, they will circulate the body at higher rates than usual. High circulatory proinflammatory cytokines are a health concern as they are linked with a number of diseases including anorexia nervosa, obesity, and cardiovascular disease (Dantzer and Kellsey 2007; Langhans 2006). As is more relevant to the present study, proinflammatory cytokines have been associated with neurological symptoms, including cognitive decline, in diseases such as Parkinson's Disease (Menza et al. 2010).

The mechanism by which immune activity interacts with the brain has been well established in the past 15 years by identifying the means by which immune cells surpass the blood-brain barrier (BBB) (Banks 2005). Cytokines passing the BBB will induce sickness behavior, which is the goal-driven, concerted effort to mount a sufficient immune response to pathogens. Much like

hunger is the drive to obtain sufficient dietary nutrients, sickness behavior is the drive to survive an attack on the immune system and maintain healthy immune function.

In animal models, sickness behavior is most commonly the elicitation of behavioral symptoms including anxiogenic or depressive behaviors, social withdrawal, decreased physical movement, and decreased exploratory behaviors. All of these behaviors allow the animal to regain normal immune function more efficiently, in an effort to minimize vulnerability of survival. For example, fever causes enzymatic denaturation to slow the pathogen replication process. While sickness behaviors are understood primarily in animal models, many of these findings have been applied to human models of clinical depression. This attribution is due to the similarity of symptoms; fatigue, anhedonia, and depressed mood are cornerstone symptoms in major depressive disorder (American Psychiatric Association 2013). A critical distinction between sickness behavior and clinical depression lies in the timecourse and causal factors. Sickness behavior is caused by immune activation, and is easily reversible upon a recovery of normal immune and inflammatory activity. Depression, on the other hand, is induced by a number of social and environmental causes, and symptoms can be indefinitely sustained.

Commonly, animal- and human-modeled methodologies differ in that animal models use behavioral operationalizations (Kirsten et al. 2015), whereas human models tend to rely more on self-report operationalizations (Eisenberger et al. 2010; Vollmer-Conna et al. 2004). No current methodology has been animal- *and* human-modeled to quantify motivation in sickness behavior, which could cause disrupts in fluidly translating the findings of one type of model to another. Adaptation of a methodology pre-existing for one of these models is ideal to bridge this gap.

B.2. Exploration and the Explore/Exploit Dilemma

A frequent animal-modeled methodology used to assess physical exploratory behavior is called the open-field test. This test places an animal in the middle of a field and, given a set amount of time, is assessed on the quantitative tendency for the animal to explore the novel space. Measures for exploratory behavior commonly include the number of head rearings, number of subsets of the field that are traversed, and total distance traversed during the set amount of time (Silva and Giusti-Paiva 2015).

A contextualization of exploratory behavior in human models lies in what is called the 'explore-exploit dilemma.' This is a dilemma that we come across on a daily basis. For example, where do you want to go for dinner: your favorite spot that makes you satisfactorily happy, or the new restaurant that could make you either incredibly happy or very disappointed?

There is differential neural activity for either an exploratory or exploitative decision. Exploratory decisions utilize more neural activity than their exploitative counterparts (Figure 1). Beyond a singular exploratory/ exploitative decision, the approach by which people handle this dilemma

across time is of interest. When given the opportunity to select between two options multiple times, participants utilize either directed or random exploration approaches. Directed exploration is the most optimal approach in terms of long-term payoff. It entails initial exploration to accumulate sufficient information from both options, then exploitation of the higher-valued option after calculating the average payoff value of each option. Random exploration, on the other hand, is the absence of strategy by randomly selecting between the two options, and can be seen as the neurologically inferior approach because of its inferential inferiority (Beck et al. 2012).

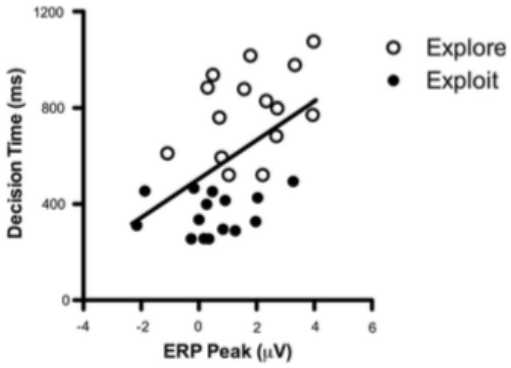

Figure 1: Event-related potential peaks as they relate to exploratory or exploitative decision-making (Hassall et al. 2013).

If one were to think of these methods computationally, random exploration is like a random binary generator, whereas directed exploration is a more complex, intricate calculation that will select for the highest value potential based on sufficient accumulated information about both options. Energetically, a directed exploratory approach is more favorable. This is because there is mobilization of the sufficient cognitive resources for the goal-directed means of obtaining enough information for both options by exploring as long as necessary, but then exploiting the higher-value option after identifying it. Random exploration, on the other hand, does not require intricate neural processing but will use more energy in the long-run. In short, the means by which directed exploration chooses to explore sparingly to rapidly identify and then exploit the better choice minimizes long-term energetic cost.

The dopaminergic pathway has also been associated with the explore/exploit dilemma due to its interaction with novelty-seeking behavior and exploratory behavior in unfamiliar places (Dantzer and Kellsey 2007). This has been attributed to genetic variations, which were shown to influence reinforcement learning style in explore/exploit contexts (Alttoa et al. 2009; Frank et al. 2009). What has yet to be found is whether there are natural, non-homeostatic physical states, such as immune activation by pathogen, that would induce enough change in neurotransmitter activity to catalyze a change in one's explore-exploit decision-making approach.

B.3. Neurotransmitter Downsignaling by Sickness Behavior

The means by which sickness behavior occurs via cytokines crossing the BBB has been well established (Banks 2005). However, little has been studied about the effects of sickness behavior on decision-making networks in the brain. While dopaminergic pathways are well established as foundational neural bases for decision-making, there have not been temporary physiological states identified that cause fluctuations in these neurotransmitters as they relate to decision-making.

Interestingly, dopamine has been associated with modulating the adaptive immune system (Basu and Dasgupta 2000; Yan et al. 2015) but it has not been associated with psychological phenomena of sickness behavior. While it is predicted that a change in striatal dopaminergic activity will be correlated with changes in psychological behavior, it is necessary to identify the degree to which these physiological and psychological changes are correlated in humans. This will help to define the active role of neurotransmitters on neurobiological higher-level processing. Further, this would identify an evolutionarily beneficial role in dopaminergic activity changes.

B.4. The Significance of Integrating Sickness Behavior-Induced Immune and Neural Activity with Exploration

The pathways by which behavior is altered in something as common as illness is functionally and clinically important, as decision-making is a pivotal aspect of daily life. Understanding the degree to which human sickness behavior impacts decision-making regions of the brain is highly important in our understanding of (1) the physiological factors of particular temporary physical states that contribute to neurotransmitter fluctuations, and (2) the gradation of behaviors and phenomenological experiences in relation to this neural activity. The ability for humans to shift their priorities both physiologically and phenomenologically would be a novel feedback cycle in sickness behavior to accommodate the goal-driven need to recover full health.

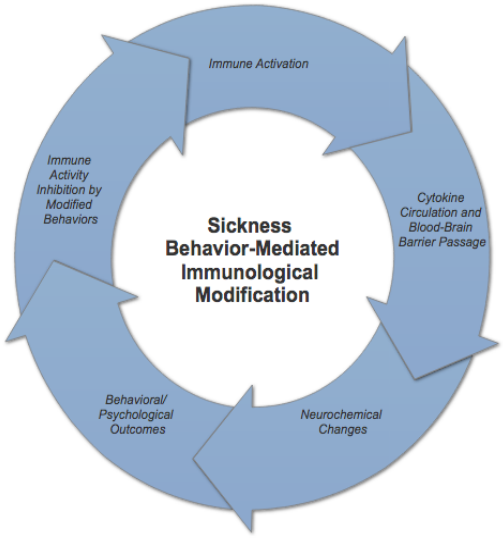

Figure 2: Peripheral immunological influence on the brain, subsequently facilitating behaviors that promote immunological activity that more efficiently regains homeostasis.

Exploration can be known as a drive for knowledge. What this work proposes is that the drive of sickness behavior in prioritizing health can supersede a person's natural proclivity toward exploratory behavior. This would identify the degree to which sickness behavior alters not only cognitive processing, but full-body (and mind) resource prioritization in functional contexts.

The knowledge of which neural regions are influenced by these decision-making tasks is well known to be the striatum and prefrontal cortex, and there are known neurotransmitters that modulate exploratory versus exploitative decision making, such as dopamine. However, there has yet to have been proposed a functional human context in which explore-exploit decision-making could be modulated within an individual. We have yet to physiologically identify the mechanisms that could facilitate the difference in exploratory approach between participants. This study attempts to identify a physiological context in which this decision-making is shifted.

C. METHODS

C.1. Study Population

C.1.1. Inclusion Criteria

Subjects were recruited through the University of Arizona psychology department as students in a psychology course. Students received credit in their psychology course for participating in the research study. These students were required (1) to have received a flu shot in the past five years, (2) to not have received the flu vaccine for the current flu season, and (3) to have been willing to come into the lab immediately after, and one week prior to or following flu shot administration. The second requirement is to ensure that the participant's adaptive immune cells are still naïve, or unexposed, to the specific strains of the virus in the flu vaccine of a particular season. Multiple exposures cause different immune responses, as the adaptive immune system functions on a more rapid timescale given multiple exposures to the same pathogen.

C.1.2. Exclusion Criteria

Because the current work is contingent on changes within participants, it is not required to exclude participants based on age, body mass index, sex, or other factors that contribute to circulatory inflammation (O'Connor et al. 2009). Individuals of all genders and ethnicities fulfilling eligibility criteria could participate. Exclusionary criteria included serious medical illness that could result in cognitive impairments, and drug or alcohol use.

C.1.3. Sample Size

Although pupillometric measures in response to a gambling simulation like the Horizon task only require 15-20 participants to have enough statistical power (Daw et al. 2006; Wilson et al. 2014), the immunological measures require somewhere approaching 40-50 people to find significant differences (Menza et al. 2010). However, because this study proposes changes within an individual rather than observed differences between subjects, it is concluded that the proposed work will find 40 people with two sessions sufficient to find significant results.

C.2. Procedures

C.2.1. Overview

A pool of undergraduate Introductory Psychology students at the University of Arizona were invited to participate in a survey that screened for inclusion criteria. Each participant then took part in two sessions, one week apart, randomized for the order of their inflammatory conditions: vaccinated, and non-vaccinated. Before one of these sessions, participants had heightened inflammation as induced by flu vaccination. In the other session, participants would not have heightened inflammatory levels. In each of these sessions, participants completed immunological assessment, psychosocial assessments, the virtual open-field test, the explore-exploit Horizon decision-making task, and pupillometric assessment during both sessions (Figure 3).

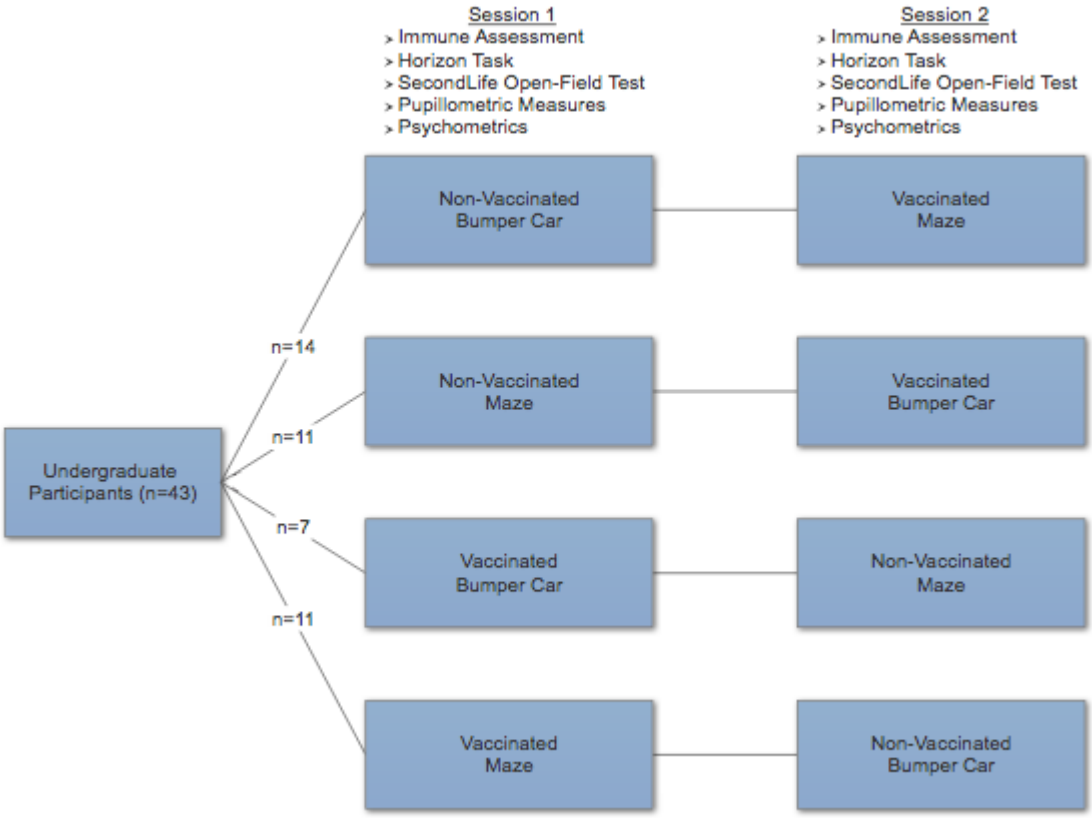

Figure 3: overview of participant tasks and conditions.

C.2.2 Immunological Markers

The proinflammatory cytokines most commonly associated with sickness behavior include IL-6, TNF-a, and IL-1. IL-6 is the most known cytokine for its relation to depressive symptoms commonly associated with sickness behavior (Lutgendorf et al. 1999).

Viruses that proliferate rapidly induce a long, drawn out recovery, thus catalyzing more identifiable sickness behavior. However, at the risk of health complications by inducing

influenza, we utilized the influenza vaccination. As an inactivated virus, this is a safer, more public-health friendly means of infection as compared to naturally contracting the flu, while still simulating viral contraction. IL-6 has been shown to be activated 24 hours after influenza vaccination (Kashiwagi et al. 2014; Ranadive et al. 2014), and thus sufficiently activates the cytokine activity that we require for the proposed work.

Diurnal fluctuations of circulatory proinflammatory cytokine levels are an important factor to control. Therefore, the testing timeframe was limited to 1:00-4:00PM, and participants schedule both sessions at the same time of day on the same day of the week, separated by seven days. The time of vaccination was recorded, as well as the time of salivary data collection.

Proinflammatory cytokines were collected by passive drool, which is a quick, minimally stressful means of collecting circulatory inflammation. Participants were provided with a Salimetrics collection container and instructed to wait until drool has pooled on or under the tongue, then to gently push drool into the container. Samples were transported in a cooler packed with -80 ice packs, then stored in a -80 freezer within an hour of data collection.

Upon completion of the study, flow cytometry by high sensitivity enzyme-linked immunosorbent assay (ELISA) was done to the drool samples to identify levels of IL-6 present. The lower detection limit was less than 0.07 pg/mL and the higher detection limit was 100 pg/mL. The mean intra-assay coefficient was 1.32%, and the mean inter-assay coefficient was 8.86%.

C.2.3. Psychosocial Measures

Participants took a series of surveys on Qualtrics, using a Mac desktop. They were first assessed by their background information, which includes ethnic, socioeconomic, and educational background. Other health information is then accumulated, including pertinent health behavior frequencies (such as exercise, smoking, and alcohol consumption), as well as health problems, reported stress for the current day, and current medications. Other scales used include:

- The Daily Stress Questionnaire
- The Perceived Stress Questionnaire
- The I/D Scale
- The Curiosity and Exploration Inventory
- The Behavioral Inhibition/Activation Scales
- Center for Epideminologic Studies Depression Scale - Revised (CES-D)
- BIS-11 - Adult
- The Tridimensional Personality Questionnaire
- Immersion and Presence in the Virtual World

C.2.4. Virtual open-field test

We have adapted the animal-based methodology of the open-field test into a virtual, human-modeled task in an effort to form a methodological cross-over. While human-modeled sickness behavior has been studied by self-report and physiological measures, behavioral measures of methodological similarity to that of animal models have yet to be done in a manner consistent with animal-based sickness behavior tasks. To identify similarity, or lack thereof, of the sickness behavior construct in a methodology that is transferrable between different populations would be highly useful in continuing the study of this phenomenon in a more consistent, standardized way.

The open-field test, previously done only as a physical maze for rats or mice, was adapted for the current study to assess similar mental constructs in humans. Virtual realms have been shown to be an effective experimental method for studying psychological traits (Blascovich et al. 2002; Schöndbrodt and Asendorpt 2011)**.** Therefore, the application of the well-established open-field test to the novel, virtual format is a logical crossover to human-modeling. This virtual setting controls for physical capability that could otherwise confound the open-field test in human models, while still assessing the construct of exploration. The current study uses a private portion of the virtual reality space in SecondLife to avoid non-experiment related interaction.

Heightened immersion can be achieved by providing interaction with other "avatars." Therefore, the present modified open-field test took use of a confederate avatar. This avatar was a researcher or research assistant simulating the role of another participant. The confederate was in the center of the maze during the maze condition; the confederate teleported to the entrance of the bumper car arena five minutes into the testing period of the bumper car arena. In this way, interaction was possible with the participant for half of the testing period. Initial exploration of the space, therefore, could not be influenced by the confederate's presence.

Confederate instructions were as follows:

1. The confederate does not initiate conversation or initiate movement.
2. The confederate is free to move through the space once the participant reaches a range in which they can engage with the confederate.
3. The confederate maintains neutral (between exploratory and stationary) movement-based behavior, to prevent influencing the participant's exploratory behavior.
4. The confederate responds to the participant's initiation of conversation in a purely reactionary fashion, in a manner reflecting the participant's level of interest and conventional social norms (i.e. "Hi, how are you?" was responded to with "I'm good, how are you?")

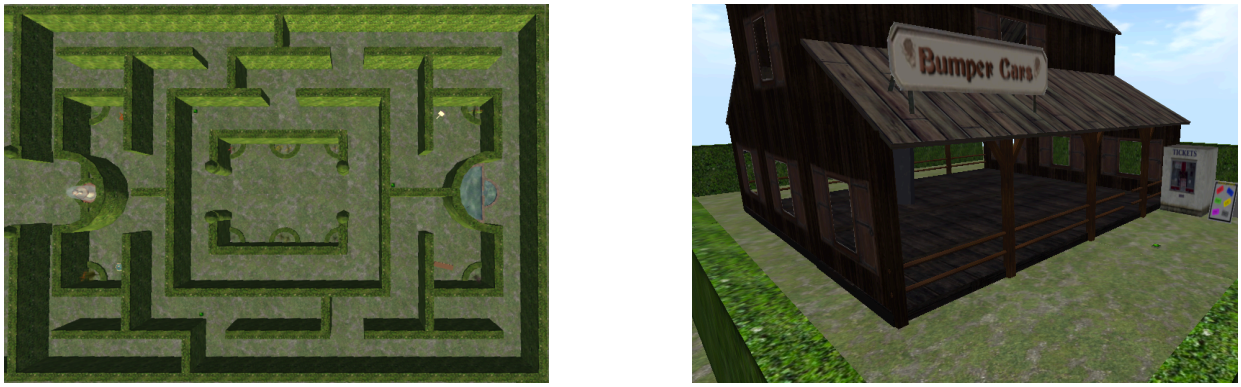

Figure 4: Second Life fields from each condition, the maze (left) and bumper car arena (b)

Participants are given access to a Mac desktop, a mouse, and a keyboard. They are given instructions on how to generally move through the space, and are provided a paper reiterating these instructions to control for memory effects. The participants are then told:

> *You will be [using virtual bumper cars/ in a virtual maze] until I come back in to tell you to stop. Feel free to do whatever you wish in the space, even interacting with other participants if you run into them during your session. We will ask you after the experiment what you can remember about the people and the space.*

During the ten minutes, participants are tracked spatially in the field. This timeframe is adapted from animal modeling, which normally utilizes approximately 80-100 rats for 3-5 minutes (Corman et al. 1967). Because the human-modeled study utilizes a smaller participant pool than animal-modeled studies (n=43), we adopted a larger timescale. To prevent testing effects, there are two conditions (Figure 4) so that during both sessions, the participant will experience a novel field. The order of these two conditions was counterbalanced. The maze condition had dimensions of 42 pixels by 60 pixels. The bumper car condition had dimensions of 42 pixels by 55 pixels. X and Y coordinates were reported by trackers approximately once every five seconds. The first ten minutes of analyzable data was used for the below calculations of exploration.

Measures of exploration included:

- Total distance traveled
- Amount of time standing still
- Number of times a gridline of the field was crossed
- Number of grids the participant traversed into
- Whether a conversation with the other avatar was initiated

Total distance was calculated through Excel as the sum of distances between two X,Y coordinate points using Pythagorean Theorem. The total distance traveled was scaled to size to account for the maze condition's relatively larger Y vector as compared to the bumper car condition. This was done by using the following scaling factor:

$$\text{Scaled Distance in Maze} = \sqrt{\Delta X + 0.92\Delta Y}$$

The amount of time standing still was calculated via Excel with a tally of the number of times the tracker identified that the participant was in the same X,Y coordinates as the last tracker record. Therefore, each tally signifies five seconds of standing in the same location.

Both the bumper car and maze conditions were split into a 10 by 7 grid to assess finer location-specific behaviors. For the maze condition, each grid had the dimensions 6 pixels by 6 pixels; the bumper car condition's grids had the dimensions 6 pixels by 5.5 pixels. A tally of the total number of times a participant passed one of these gridlines (passed from one grid into another) was summed as the number of grid traversals. This can indicate directionality, and is associated with the measure for total distance traveled. Lastly, the total number of grids intersected out of 70 that each participant traversed into was measured. This is simply a tally of how many grids were intersected at any point in the testing period. Lastly, research assistants recorded whether a conversation was initiated during the ten-minute period, and conversation transcripts were recorded, as well.

C.2.5. Explore-exploit Horizon Task

This task replicated Dr. Robert Wilson's computer-based Horizon task (Wilson et al. 2014) through Matlab that simulates the explore-exploit situation of gambling as a selection between two slot machines. Each game presents two slot machines, in which one has a higher mean payoff than the other. One slot machine was either a mean payoff of 40 or 60, and the other slot machine's mean was based on the former, such that the mean difference between the slot machines was either 4, 8, 12, 20, or 30 in a counterbalanced design for mean difference and higher-payoff slot machine. Each option paid between 1 and 100 points in a Gaussian distribution with a fixed standard deviation of 8.

Participants first underwent an explicit tutorial on the instructions for the game. These instructions delineated that one slot machine is better than the other in each game, and that the goal of the game is to win the highest amount of points. They then played four blocks of forty 'games;' the components of one game are shown in Figure 5. A game can have either one or six decisions in it after four forced–choice trials. The selection and outcome history stay on the screen for the duration of the game, while the unselected slot machine for each decision displays an "XX".

Forced-choice trials, where the participant is told to select a particular slot machine, control the amount of information provided by the two options to assess one's tendency to explore or exploit in relation to the amount of knowledge given for these options. Determining a choice as exploratory or exploitative is decided based on the 'observed mean.' This entails a calculation of the estimated mean payoffs of each slot machine, based on the information presented by the preceding trials. Whether the participant actually selected the higher or lower value slot machine, in terms of observed mean, is considered the exploitative or exploratory option respectively.

The information presented about each slot machine was either equal (with two point values presented by each slot machine) or unequal (with 1 point value presented by one slot machine and 3 point values presented by the other slot machine). Directed exploration was assessed as the probability of selecting the option with more information in the unequal information condition (i.e. selecting the slot machine that had three points presented in the forced-choice trials). Conversely, random exploration is a measure of the probability of a participant selecting the low mean reward option based on the information presented to the participant thus far.

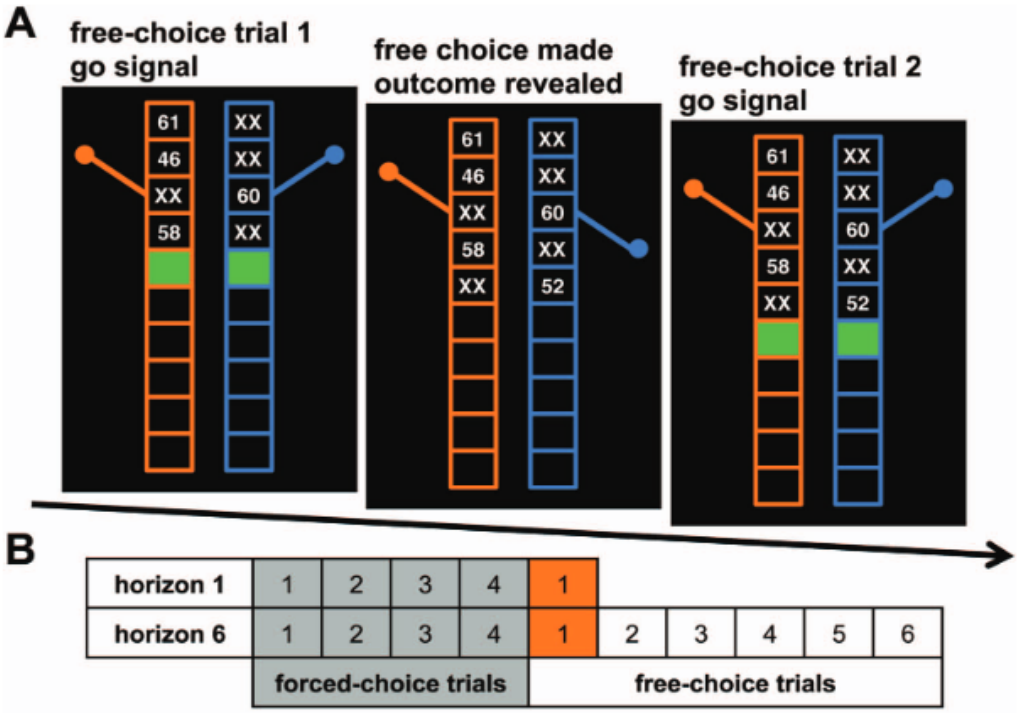

Figure 5: (A) A screen shot simulation of a decision's progression in a horizon-6 game on the first free-choice trial. (B) Schematic representation of a horizon-1 and horizon-6 game (Wilson et al. 2014)

C.2.6. Pupillometric Measures

Common eye tracking software and techniques for explore-exploit dilemma are being used in the current study. The EyeTribe, an easily programmable pupillometer that utilizes infrared illumination, is being used to assess various measures. At a sampling rate of 30Hz, the pupillometer accumulated data on: pupil diameter, the location on the screen of pupil fixation, and head movements. Participants were provided a chin rest with forehead stabilization and the pupillometer was situated 2 feet from the participant, directly under the computer monitor.

After a calibration period, participants were told to daydream while still looking forward at the computer screen. It is at this point that a blank screen appeared for the participant and blink rate was assessed for five minutes. Blink rate was determined as the average number of blinks per minute over the five-minute period. Five minutes is a standard amount of time for accurate assessment of average blink rate. The pupillometer additionally took measures during the Horizon Task, to assess pupillometric data during exploratory or exploitative decisions. Because dopaminergic levels have been associated with changes in blink rate (Taylor et al. 1999), within-subject changes in blink rate can be calculated to identify changes in striatal dopaminergic levels.

D. RESULTS

D.1. Demographics

Sixty participants enrolled in the present study upon meeting inclusion criteria. 8 were excluded for not taking part in a second session; 6 were excluded due to virtual-reality open-field test artifacts; 3 were excluded for salivary sample artifacts. Forty-three participants with complete data were included in the following analyses below. The sample population was an average of 19.22 years of age, 67% female, and 67% Caucasian (Table 1). There were no differences between the number of participants who had the bumper car or maze first under the vaccine and no vaccine conditions ($X^2 = 1.23$, $p < 0.36$).

Sex	**N**		
Male	14		
Female	29		
Race	**N**		
Caucasian	29		
African American	4		
Asian American	4		
Native American	3		
Other	3		
Age	**Mean**	**Range**	**Standard Deviation**
	19.22	17.82-27.37	1.69
Body Mass Index	**Mean**	**Range**	**Standard Deviation**
	23.26	17.23-31.32	3.39

Table 1: Sample demographics

D.2. Vaccination-Induced Differences Within-Subject

D.2.1. Circulatory Inflammation

IL-6 rose significantly on the day of vaccination compared to a non-vaccine day, when accounting for BMI ($F(1, 41) = 4.57$, $p < 0.04$).

D.2.2. Psychological Assessment

When vaccinated, participants reported significantly less perceived stress on the Perceived Stress Scale ($t(42) = -2.52$, $p = 0.02$). Additionally, the BIS 11 - Adult identified diminished cognitive attentional impulsivity upon receiving vaccination ($t(42) = -1.97$, $p = 0.03$, one-tailed).

D.2.3. Virtual Reality Open-Field Test

Participants traveled significantly less, stood still longer, and crossed more subfield barriers in the virtual reality field when vaccinated. Figure 6 provides a visualization of the coordinate output from participant movement in the open-field test; total distance traveled is therefore a measure of the length of the line. Participants additionally initiated conversation less frequently when vaccinated during their time in the virtual reality task (Table 2).

	t	df	Sig.
Total Distance Traveled	-2.94	42	< 0.01
Time Standing Still	3.08	42	< 0.01
Gridline Traversals	-2.78	42	< 0.01
Total Grids Intersected	0.36	42	0.72
Conversation Initiation *	-1.71	38	0.05

Table 2: SecondLife Open-field test behavioral measures within subject as measured by a paired samples T-test. * one-tailed

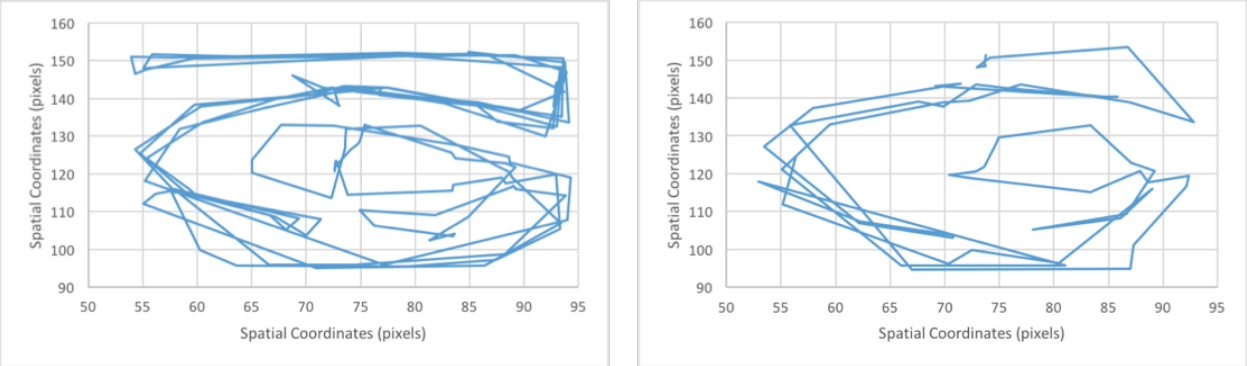

Figure 6: Plots demonstrating individual participants in the same virtual environment when non-vaccinated (left) and vaccinated (right). Individual subjects represented were matched for age, sex, race, and BMI.

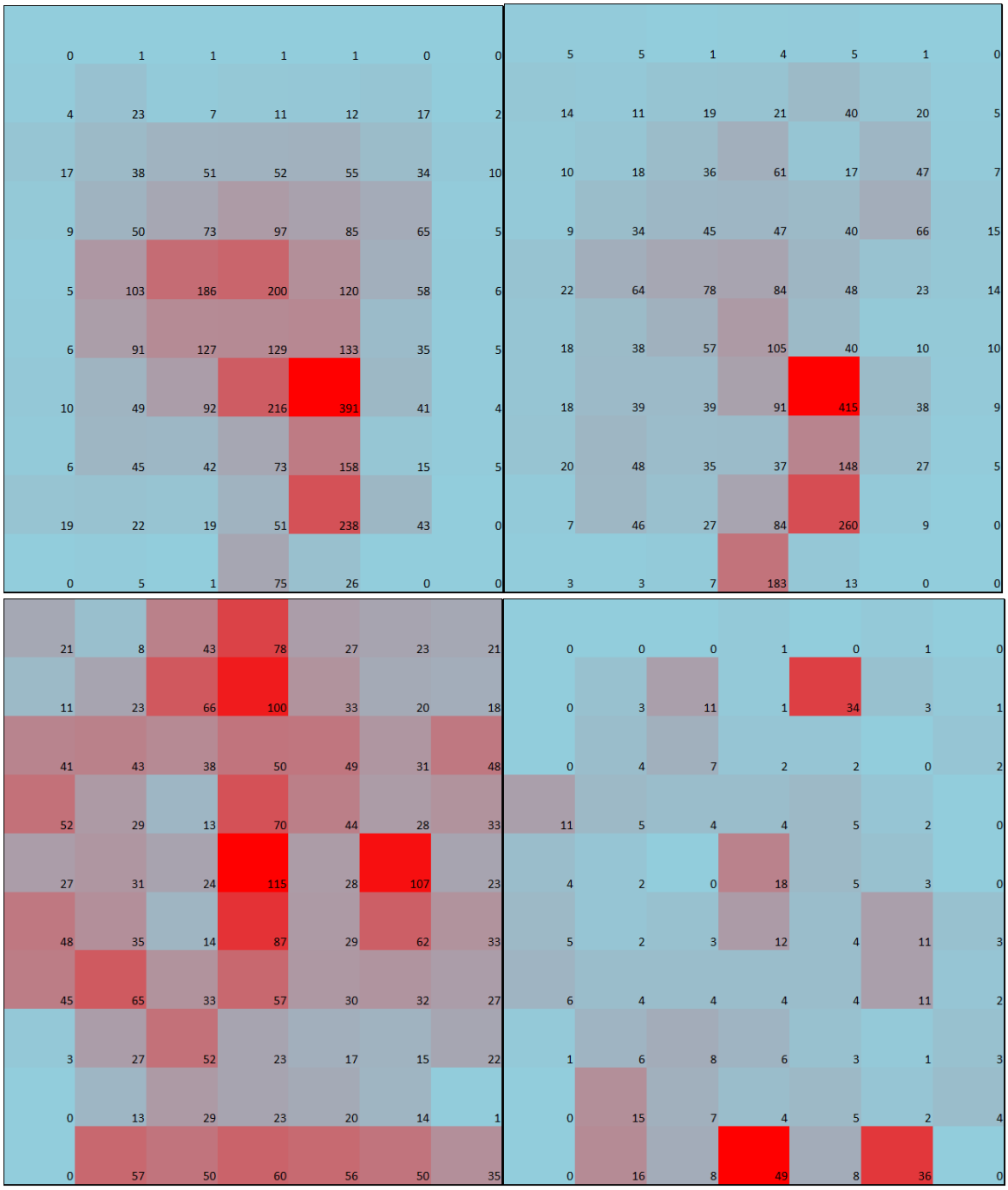

Figure 7: Heat map of time investment during the virtual open-field test in a bumper car while non-vaccinated (top left) and vaccinated (top right), and the maze while non-vaccinated (bottom left) and vaccinated (bottom right).

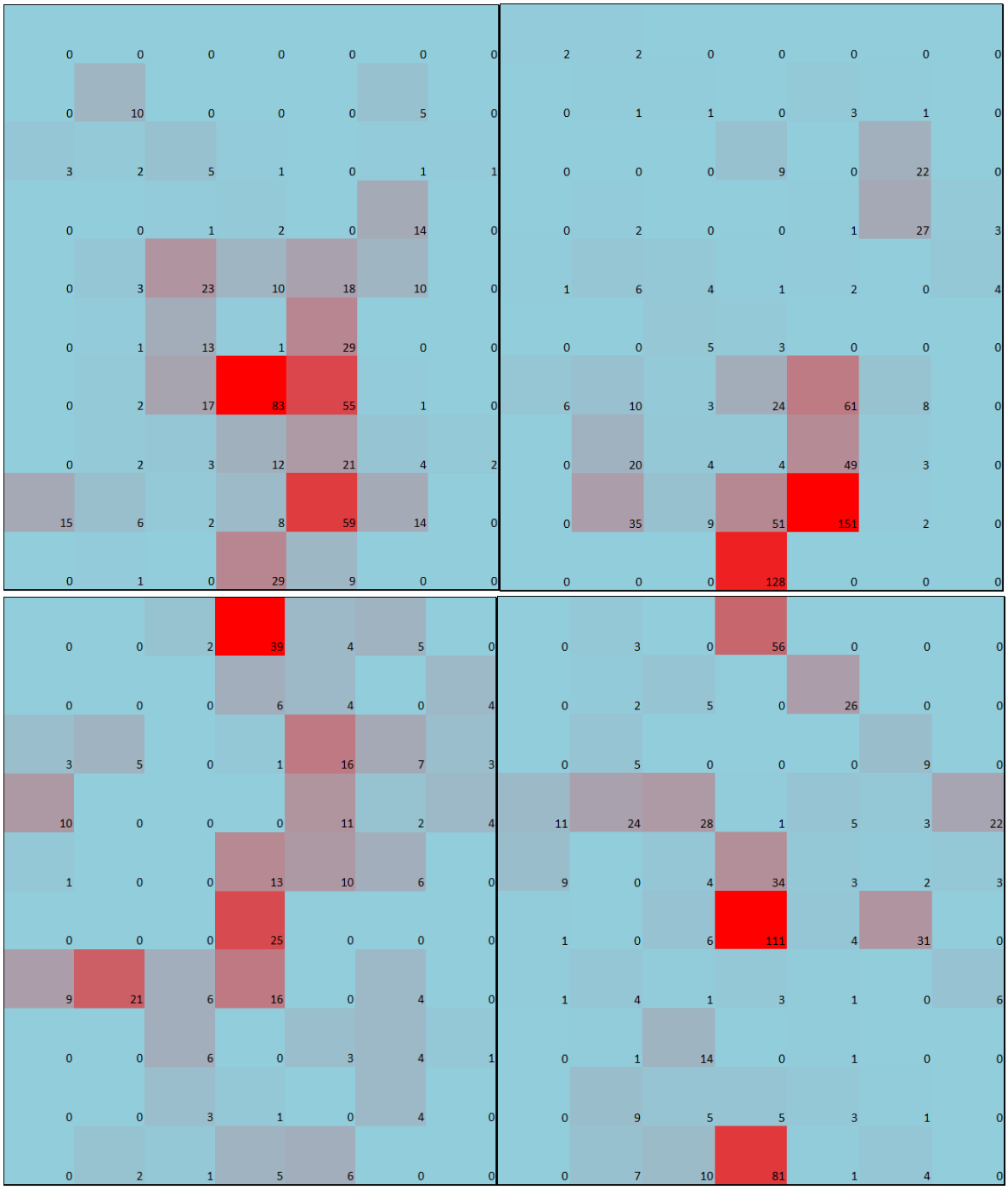

Figure 8: Heat map of time spent standing still during the virtual open-field test in a bumper car while non-vaccinated (top left) and vaccinated (top right), and the maze while non-vaccinated (bottom left) and vaccinated (bottom right).

D.2.3. Pupillometry

Blink rate as measured in the five-minute baseline period did not yield a significant difference upon receiving vaccination ($t(33) = 1.50$, $p = 0.14$) . In addition, the change in blink rate between vaccination and non-vaccination sessions was not correlated with changes in total distance traveled ($r = -.10$, $p=0.59$), or with change in IL-6 ($r = -0.12$, $p=0.49$).

D.2.4. Horizon Task

Participants' directed exploration was not significantly different on the day of vaccination compared to the non-vaccinated day ($t(38) = 0.13$, $p = 0.90$). Similarly, participants' random exploration was not significantly different on the day of vaccination compared to the non-vaccinated day ($t(38) =-1.02$, $p = 0.32$).

D.3. *Inflammation predicts open-field test behavior*

To test whether inflammation predicted the exploratory behavior in the virtual open-field test, a regression model predicted the change in total distance traveled from non-vaccination to vaccination, with three predictors: the change in IL-6 from non-vaccination to vaccination, BMI and the interaction IL-6 change with BMI. The model was significant ($F = 3.01$, $p = 0.04$), and all three predictors were significant. In a similar model using the change in gridline crossings, the model was also significant ($F = 3.58$, $p = 0.02$), and all three predictors were significant. However, predicting the change in total gridlines crossed was not significant ($F = 1.41$, $p = 0.25$), nor was predicting the change in time standing still ($F = .82$, $p = 0.49$).

E. DISCUSSION

E.1. Psychological Implications of Vaccine-Induced Sickness Behaviors

Changes in self-report measures of perceived stress and impulsivity upon vaccination provide support for the notion that the pre-frontal cortex is being implicated. A change in impulsivity is advantageous in sickness behaviors, as diminished impulsivity falls in line with the behavioral changes seen in the open-field test: decreased motivation for novelty, be it more virtual space or a new cognitively complex challenge, allows the individual's energetic prioritization to emphasize immunological activity.

Decreased perceived stress upon vaccination seems counterintuitive to the notion of sickness behavior, as one of its benchmark symptoms is anxiety. Fundamental attributions to stress and anxiety, however, may allow the important distinction to be better understood. Stress is due to a current threat to homeostasis; it is an event that the person can identify as the cause of non-homeostatic behavior. Anxiety, on the other hand, demonstrates similar symptomology but lacks an attributive cause. For instance, one can be anxious about an exam that is happening the next day, or one can be stressed upon being handed an exam. While anxiety may be heightened by inflammation-induced sickness behavior (Reichenberg et al.), the perceived stress of one's

physical day-to-day environment is a distinctly different construct. This diminished stress perception may actually speak to the anhedonia that is additionally a hallmark in human sickness behavior. Day-to-day situations may not seem stressful because of a lack of motivation to put effort toward resolving that situation. While one demonstrating sickness behaviors may demonstrate generalized anxiety, actual daily situations and activities may not directly contribute to the perception of this anxiety.

E.2. Ineffect of Vaccine-Induced Inflammation on Dopamine and Decision-Making

While dopaminergic changes were not significant in the present study, the behavioral and psychological results found here support our claim that dopamine appears to be importantly implicated in sickness behaviors. This insignificant result can be attributed to one of many causes: a too small sample size, insufficient immunological activation by our vaccination method, and methodological limitations of dopamine assessment included. The complexity of the human brain brings with it a myriad of checks and balances to prevent small physiological events from causing gross homeostatic changes. While inflammation is known to be implicated in some psychological diseases (Simmons and Broderick, 2005), it is possible that the presence of inflammation alone is not sufficient to induce neurochemical changes without further inducers presented to the system. Additionally, as blink rate is an indirect, albeit reliable assessment of striatal dopamine, little research has been done as to the precision with which slight changes within a person could be assessed by a gross assessment of blink rate. Insignificant decision-making changes can also be implicated by the above limitations of the present study. With the complexities of decision-making, it would be advantageous for human behaviors to not be swayed by a relatively small insults to the body, as is an influenza vaccination.

The pupillometric output reports a number of variables to ensure normal function and data collection, including the dropout rate: the rate at which data from the pupillometer had been ‘thrown out’ from the program’s final analysis. Interestingly, upon vaccination, there was a higher dropout rate from the pupillometer ($t(33) = -2.01$, $p = 0.05$). This data could be indicative of other pupillometric-related changes. For instance, closing one’s eyes for long amounts of time or moving one’s head out of the headrest could be attributed to a heightened dropout rate. A video of participants’ faces during the pupillometric assessment would be a suggested future step in better understanding the behavioral change causing this increased dropout rate.

E.3. Virtual Open-Field Test Methodology and Sickness-Behavioral Implications

The virtual adaptation of the open-field test is a novel method which can be used to assess not only exploration, as it does in its animal-modeled counterpart, but additionally provides another means by which motor-independent assessment of motivation can be tested in humans. The methodologies done in the present paper present promising findings as to this metholodogy’s ability to provide a valid report of motivation. The possibilities in expanding assessment through this simply administered task are widespread. For instance, eye tracking as done in other tasks of

the present study could bring to light other potential mechanisms, such as studying eye fixations or saccades (Phillipou et al. 2014).

The present study brought to light a number of important variables that require control. For instance, a standardized tutorial on how to use the virtual space would prevent participant distraction (ie. reading the written instructions during the measured timeframe). Controlling visibility, preferably through a means such as virtual reality goggles, would have the dual benefit of increased immersion as well as better precision in and external validity to pupillometric analyses.

The cytokine IL-1 has been described to influence memory in hippocampal regions (Banks 2002). IL-1 should play an important role in the proposed tasks. IL-1 is correlated with spatial memory (Broderick et al. 2002), which is relevant to the open-field test since spatial memory with the aide of provided landmarks could be a mechanistic factor influencing performance on this task. Also, IL-1 has been linked with depressive behaviors in rodents (Simmons and Broderick 2005). It is therefore a promising beginning to understanding the complex physiological changes that are stimulated in and by the immune system upon simple influenza vaccination.

E.4. Clinical Implications

The monoaminergic hypothesis of clinical states that diminished dopamine, norepinephrine, and serotonin give rise to the depressive symptoms that have heterogeneous attributes, including genetics, environment, or life experiences. Due to the overlap in symptoms for sickness behavior and depression, it is useful to identify the biochemical distinctions between depression (known for its diminished striatal dopamine levels) and temporary states of sickness. By identifying key distinctions between these two pathways, interventions can better target the maladaptive mechanisms for such psychiatric disease.

Further, understanding the effects that inflammation can have on an individual can allow clinicians and psychologists to understand mechanistic underpinnings for phenomenological symptoms. This could allow healthcare professionals to more holistically provide quality of life to patients, beyond merely treating disease. Additionally, human disorders presenting such behavioral and psychological symptoms could be studied under a more interdiscplinary scope, to see whether similar immunological mechanisms are eliciting such experiences.

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