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<u>04/26/2009</u> Date Exclusive Challenges in Modeling Psycho-Social Stress:

The Cyberball Experience

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Department of Neuroscience and Behavioral Biology

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#### Abstract

Exclusive Challenges in Modeling Psycho-Social Stress:

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#### By Marissa Rene Krimsky

Previous literature suggests that an interactive computer game called Cyberball can be used to elicit central nervous system stress and distress. We postulated that Cyberball could similarly activate the peripheral stress response both initially and upon repeated testing. This was measured through heart rate variability, cortisol, and momentary mood reports. 18 subjects were divided into two groups, ostracized and control. Subjects were tested one at a time in either the control or experimental group. The Profile of Mood States (POMS) evaluated general levels of distress at baseline, immediately following the Cyberball game, and 30 minutes after concluding the Cyberball game. Heart rate variability was collected throughout the game and two cortisol samples were collected before and after the game. All subjects played two rounds of Cyberball in each session the ostracized subjects returned after 2 weeks for a second session. There were no significant findings between the delta values of the control group versus the excluded group for cortisol levels, THM, RSA, or POMS. The larger mean POMS delta value of the control group suggests that the mood state of the excluded group improved at a lower rate the control group. The only significant result from this task was reported invisibility scores. This study suggests that the Cyberball task may not be powerful enough of a stressor to activate peripheral stress responses.

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# **Table of Contents**

Introduction1
Materials and Methods7
Procedure7
Figure 1: Cyberball screen
Figure 2: Procedural template
Results15
Results Session 115
Results Session 2
Discussion
Table 1: Mean Delta values from baseline to post-Cyberball
Table 2: Descriptive Statistics
Table 3: Excluded Group (Session 1): Stress Variable Correlation Values
Table 4: Excluded Group (Session 1): Stress Variables and Psychological Assessment Correlation         Values       27
Figure 3: Mean POMS score by time (values transformed)
Figure 4: POMS score trends28
Figure 5: POMS score trends by individual control subjects
Figure 6: Cortisol score trends by mean
Figure 7: RSA score trends by mean
Figure 8: THM score trends by mean
Figure 9: Session 2 and Session 1 POMS mean (values transformed)
Figure 10: Session 2 and Session 1 Experimental group cortisol means
References

# Introduction

Social exclusion has been repeatedly posited to engender negative emotions in humans because of the survival advantages it has posed over the long period of human evolution.<sup>1234</sup> From an evolutionary standpoint, it is understood that certain social behaviors such as courting, mating, offspring care, cooperation, and distress calls put selective pressure on an individual, conferring a need to belong in order to survive.<sup>5</sup> In this regard, being included or rejected within the complex web of social group dynamics is theorized to be fundamental to maintaining both psychological and physiological health.<sup>6</sup> The overt and implicit pressures of social acceptance affect not only long-term health, but may engender significant, acute emotional distress when acceptance is denied. Indeed, evidence suggests that the pain of social exclusion elicits a neural response similar to physical pain, implying that the neural circuitry for social pain has gradually co-opted that of physical pain.<sup>7</sup> Studies utilizing experimental models of social exclusion create both psychological distress as evidenced by subjective reports of fear and anxiety<sup>8</sup>, and a physiological stress response, objectively characterized as increases in heart rate and circulating cortisol<sup>910</sup>. Finally some studies have also found a correlation between psychosocial stress and activation of innate immune inflammatory responses. These broadly deleterious effects, stemming from an individual's perception of social exclusion, clearly have potential to promote widespread physical and mental pathology.<sup>111213</sup>

Consistent with the influence of social interactions on health and wellness, methods of prevention and intervention for socially stressful situations have taken center stage in a variety of modern medical disciplines. A preliminary step in being able to investigate social stress as an isolated and controlled experimental variable is to develop standardized laboratory social exclusion paradigms that reliably activate the stress response. One of the most popular experimental models of social stress is the Trier Social Stress Test (TSST), which has proven to be a robust stressor in laboratory settings<sup>14</sup> TSST experiments have shown increases in heart rate and cortisol as well as subjective reports of psychosocial stress<sup>15</sup>. Additionally, significant elevations in concentrations of adrenocorticotropic hormone (ACTH), growth hormone (GH), and prolactin<sup>16</sup> have also been documented. The TSST has also been repeatedly demonstrated to activate innate immune inflammatory pathways.<sup>17</sup>

While experimental paradigms such as the Trier Social Stress Test have been consistent in inducing physiological stress reactions, it is not a technique that is easily translated into the brain scanner environment, pointing to the urgency of identifying scanner based paradigms for social exclusion that are robust enough to activate the peripheral stress response.<sup>18</sup> Moreover, few, if any, stressors have been evaluated in longitudinal studies and results have been inconsistent.<sup>19</sup> Longitudinal designs are considered the gold standard for any empirical study that aims to investigate the effectiveness of behavioral and pharmaceutical interventions on psychosocial stress. Because of this, in the current study we sought to examine whether a widely used fMRI-based social exclusion paradigm reliably activates the peripheral stress response and does so repeatedly in a longitudinal design.

Cyberball is a computer ball game that appears to participants to be online. Participants can either be told that they are playing against real players or a computer algorithm; however, the other players are always pre-programmed. The game includes cartoon players alongside of photographs and names; both of which may be changed for the particular study. The number of players and time increments are established by the experimenter. There are two conditions for the game, either inclusion or exclusion. If the inclusion condition is selected the participant receives ball tosses from the other players during the entire time allotment. If the exclusion option is selected, the participant stops receiving the ball from the other players at some point during the game.

Previous studies report that the exclusion round during Cyberball is indeed a simulator of social exclusion, as evidenced by self-reports of distress. Distress was marked by a four needs criterion, developed in the original Cyberball experiment.<sup>20</sup> These needs included reported feelings of belonging, control, self-esteem, and meaningful existence. In addition, to producing objective emotional distress, the Cyberball task has also been shown to activate several brain areas, including the dorsal anterior cingulate cortex (dACC),<sup>2122</sup> which is known to be more active in times of conflict and has been linked to activation of the autonomic nervous system, which—in turn—plays an important role in activating innate immune pathways in response to stress.<sup>23</sup>. These findings suggest that exclusion caused by Cyberball may have physiological stress effects.

Furthermore, there have been multiple sociological studies using Cyberball to evaluate psycho-social parameters and their relation to ostracism. To date, evidence demonstrates that the level of reported ostracism caused by Cyberball was correlated to social status of gender and educational levels<sup>24</sup>. Cyberball has even been used to predict learned prejudice in children against particular groups<sup>25</sup>. Similar to these findings, individuals playing Cyberball even report distress when they are excluded by a despised group, such as the KKK<sup>26</sup>. Furthermore, studies have shown that exclusion by Cyberball correlated with tendencies associated with anti-social behavior,<sup>27</sup> as well as behaviors of mimicry of the excluding members by the excluded

individual.<sup>28</sup> In another study, participants who were excluded reported colder room temperatures than those who were included<sup>29</sup>. Importantly, self-reported distress was endorsed even if participants were aware Cyberball was an algorithm designed to exclude them. <sup>30</sup>

Feelings of exclusion caused by playing Cyberball have also been linked to fluctuations in emotional states. In one model, feelings of jealousy caused by ostracism from a sexually desired partner were correlated with higher activation in the left frontal cortex, as measured by Electroencephalography (EEG).<sup>31</sup> A possible determinant of how in control individuals feel during the Cyberball game depends on personality types; empathizers report feelings of lesser control than systemizers.<sup>32</sup> If inclusion was linked to a monetary payoff, subjects were more frustrated by being ostracized within the Cyberball game; however, there was also guilt associated with being over included.<sup>33</sup> Similarly, those with higher anxiety attachment and higher baseline pain thresholds report higher pain thresholds to exclusion than to controlled conditions<sup>34</sup>. Socially anxious individuals are also slower to recover stability with the four needs.<sup>35</sup> Lastly, exclusion during Cyberball has been demonstrated to lead to lower self-restraint, a marked response to stress.<sup>36</sup>

In the aforementioned studies, the reports that confirmed feelings of exclusion and distress caused by Cyberball were related to, or based upon, the four needs criterion questionnaire taken from William's original Cyberball experiment.<sup>37</sup> There are some concerns with this methodology. Participants may have been responding in a perceived socially appropriate manner rather than reporting how they actually felt due to cues of ostracism and not belonging in the questionnaires. Prior studies have repeatedly shown that Cyberball causes individuals to report feelings of distress via the four needs criterion. However, a lack of more

rigorous psychological and physiological assessments of the actual effect of being ostracized limits the certainty of conclusions one can draw in terms of how robust a stressor the Cyberball task really is.

Due to a significant gap in the literature, it is unclear if Cyberball creates responses of psychological and/or physiological stress. No published studies have assessed changes in cortisol levels, heart rate variability, or acute feelings of stress, anxiety, and depression resulting from the task. If exclusion caused by the Cyberball task is psychosocially stressful, one might expect it to increase cortisol levels, decrease vagal tone, and increase reported momentary stress, anxiety, and depression. These assessments are crucial to confirm Cyberball as a social stressor comparable in effect and magnitude to better characterized psychosocial stressors such as the TSST, which has been shown to exhibit strong stress induced psychological and physiological responses.

Common physiological markers of stress include cortisol levels and heart rate variability (HRV). Cortisol, the end product of corticotrophin-releasing hormone (CRH) production and release is the primary effector of the hypothalamic-pituitary-adrenal (HPA) axis. In addition to the HPA axis, the sympathetic nervous system is a primary stress response pathway.<sup>38</sup> High frequency in heart rate variability (HRV) is believed to reflect parasympathetic nervous system activity. Low frequency of heart rate variability has a less obvious relationship with autonomic activity and has been posited to reflect both sympathetic activation and vagal activity. Decreased vagal tone has been correlated with stress and depression.<sup>39</sup> Respiratory sinus arrhythmia (RSA) (which is equivalent to high frequency HRV and is a measurement of the respiratory effect on pulse) is lower in aerobically trained individuals, indicating that a lower RSA correlates to faster

recovery from stress<sup>40</sup>. The Traube-Herring-Mayer (THM) band, associated with low frequency, is also a marker of stress<sup>41</sup> but its exact relationship is still controversial. Evidence suggests that changes in cortisol levels and heart rate variability reflect physiological stress; therefore, collecting data regarding these markers may confirm the validity of Cyberball as a psycho-physiological stressor.

A further weakness regarding the applicability of Cyberball is that there is no data regarding whether the task can be used in a longitudinal manner. Repeating Cyberball seems simple; however, because it involves deceptive social exclusion, to our knowledge, there are no published studies evaluating the utility of Cyberball in a longitudinal design.

We postulated that Cyberball would activate the peripheral stress response both initially and upon repeated testing. To test this postulate we examined whether Cyberball increased cortisol release, affected HRV and induced mood changes, as measured by the Profile of Mood State (POMS). Secondly, we sought to evaluate the Cyberball task for repeatability within the same subject group, seeking to examine whether the task would lead to equivalent stress system activation upon re-testing. In order to validate Cyberball as a stressor we compared a group of individuals who were excluded during Cyberball with a control group that only experienced social inclusion. As an adjunct to these experiments, we sought to examine individual differences in mindfulness, reported spirituality, depression and anxiety, and levels of psychopathy, and to investigate if these individual differences were related to subjective and physiological reactivity to Cyberball.

## **Materials and Methods**

Recruitment and Randomization

Participants were recruited from the Emory University campus using fliers. All subjects were screened and excluded if they had any previously diagnosed medical or mental health issues (including substance abuse), or if they used any psychotropic medications or medicines that might affect autonomic activity within the previous year that might affect the autonomic nervous system. This information was collected through self-reported screening forms. Upon enrollment, subjects were randomly assigned to either standard Cyberball (with social exclusion) (n=10) or a control condition without social exclusion (n=8). In the ostracized (i.e. excluded) group, there were 6 males and 4 female participants, the age range fell between 20-27. In the control group, there were 5 males 4 female participants, the age range fell between 18-28.

## *Compensation*

Upon completion of the experiment, participants were compensated a monetary value of approximately 45USD for participating in this experiment. The control group was awarded 10USD for their participation.

## Procedure

Prior to entering the laboratory, a written consent form was sent to the subjects. This was collected before beginning the study. The following measures were taken to normalize the circadian rhythm of cortisol. Subjects were asked to abstain from use of over-counter non-steroidal anti-inflammatory agents or aspirin beginning 48 from the initial study.

Subjects were tested one at a time in either the control or experimental group. After entering the laboratory, the first salivary cortisol samples were collected at T=0. The subject's photograph was taken and uploaded into the Cyberball program. Subjects were then administered a shortened version of the first Profile of Mood State (POMS). The POMS was given on three occasions to evaluate levels of distress. The Profile of Mood States (POMS) evaluated general levels of distress at baseline, immediately following the Cyberball game, and 30 minutes after concluding the Cyberball game. It was collected in both the first and follow-up experiments.

Subjects also completed the Kentucky Inventory of Mindfulness Skills (KIMS)<sup>42</sup>, the Spiritual Meaning Scale (SMS)<sup>43</sup>, Psychopathic Personality Inventory (PPI)<sup>44</sup>, and the Depression Anxiety Stress Scale (DASS-42)<sup>45</sup>. It took an average of 15 minutes to complete these scales (See Figure 2 for ordering procedure).

Subjects were introduced to two confederates that were members from our laboratory. Subjects were told they would be playing an interactive game online with these individuals (the confederates). Confederates and participants shook hands and then the confederates were led away by separate lab members who claimed to be administering the test to the confederates. Two confederates, a male and a female, were used for all subjects to account for biases of sex. In fact, subjects were actually playing a computer algorithm.

The online game is the Cyberball task.<sup>46</sup> A Biolog, a device for measuring heart rate variability, was attached to the subject. In the game, the participants saw their photograph, name and a cartoon image of a hand, which represented them in the low center of the screen. In the upper right and left portions of the screen were photos, names, and cartoons of the confederates (See Figure 1). The Cyberball game was set for the standard amount of throws per game with the

computer players (represented by confederates) waiting a variable time between 0.5 and 3 seconds before returning throws. All subjects played two rounds of Cyberball in each session. There was a 1 minute break between games. The inclusion and exclusion round each ran approximately 2 minutes and 20 seconds.



Subjects in the control group were included in both rounds of the game, whereas subjects in the experimental group were excluded after approximately the seventh throw on the second round. The experimenter left the room during the game so that the subject was in the testing room alone. Altogether the Cyberball game took approximately 10 minutes. Immediately upon completion of the game, subjects were given a second POMS questionnaire. Next, subjects were instructed to sit quietly without falling asleep for 30 minutes and a second salivary cortisol sample was then a third POMS was completed.

# Figure 1: Cyberball screen

Control participants then were given an assessment of the Cyberball game – the Cyberball questionnaire. The questions based on the four needs criterion and taken from William's original experiment were: *How much do you feel you "belonged" to the group, How true is the statement: 'Life is meaningless'?, How much did you feel "invisible" during the online game?, How true is the statement: 'I am in control of my life'.* They were debriefed, thanked for their participation, and compensated.

Following the final collection of cortisol in the first session, subjects in the ostracized group were told the following script:

"We are just starting up this study and we have been having a little bit of technical trouble. It seems like your computer went off-line during the second game, because the other subjects said they were trying to throw the ball to you and it wouldn't work. You will get another chance to play with two new people when you come back in two weeks, and we hope that the game will be fixed by then."

The experimental group was requested to return in two weeks time for session 2. The control group existed to test the validity of Cyberball as a stressor; therefore it was not necessary to repeat the task within the control group. If subjects returned for a follow-up, they were scheduled during the same time of day, to control for diurnal variations in cortisol. Because several of the psychological assessments are long-term oriented, the KIMS, SMS, PPI, and DASS-42 were eliminated during the post-experiment. The POMS, however, was included during both pre and post experiments. During session 2, two new confederates were introduced to the subject. Also, prior to starting the Cyberball game, subjects were told the following.

"During the first couple of days of the experiment we were having a lot of trouble with our computers going offline in the middle of the games. We have fixed all of the problems, so no computers are going offline anymore."

Upon completion of round 2, a second cortisol saliva sample was collected as well as a third POMS. Subjects were given the Cyberball questionnaire. Finally, ostracized subjects were completely debriefed regarding the experiment and compensated.



#### **Figure 2: Procedural template**

**Physiological Instruments** 

Cortisol was collected through salivary samples. A baseline sample was collected before the administration of the questionnaires. A secondary sample was collected 30 minutes following the completion of Cyberball. 3mL of saliva was collected in 15 mL poly-propane test tubes and frozen immediately after the completion of the entire experiment. These samples were kept frozen for between 1-3 weeks time and batch processed with a 96 well Salimetrics Salivary Cortisol Kit following standard procedure included with the kits. Coefficient of variation for cortisol radioimmunoassay in saliva was between 0 and 26.9 percent.

A 3991x/2-EIS Biolog ambulatory data recording system (UFI, Morro Bay, CA), which records cardiac interbeat intervals, was hooked up and activated shortly after the participant met the confederates. For purposes of analysis, an identifying mark was added to the biolog when the subject began each round of Cyberball. Heart rate variability was edited in accordance with the Porges method in CardioBatch software at the Brain-Body Center, University of Illinois at Chicago.<sup>47</sup> The intervals were established based on the detection markers placed at the onset of each game trial and then averaging these intervals.

The HRV data was averaged into intervals due to the variability of onset of the stressor. The three intervals were *baseline* (the time period that the individual is in the experimental room reading the instructions), *game 1* (the time period during a regular non-excluded game), and *game 2* (the time period during either an excluded or non-excluded game dependent on the condition).

#### **Psychological Instruments**

The 30-item Profile of Mood State (POMS) identifies positive and negative states of affect using a 1-5 Likert scale.<sup>48</sup> In accordance with standard scoring methodology, distress was calculated by adding five subscales: *tension-anxiety, depression-dejection, anger-hostility, fatigue-inertia,* and *confusion-bewilderment* and subtracting scores on the *vigor-activity* subscale.

To evaluate whether individual differences in mindfulness, personality, and mood were associated with exclusion, before playing Cyberball participants administered the following questionnaires: DASS-42, KIMS, PPI, and SMS.

The Depression Anxiety Stress Scale (DASS-42) was administered to determine more universal measures of depression, anxiety, and stress than the POMS; it was administered only during the first experiment. The three sub-scales, *depression*, *anxiety*, and *stress* were calculated by summing the respective questions in accordance with standard procedure and were left separate in our analysis.

The Kentucky Inventory of Mindfulness Skills (KIMS) score was calculated by summing the four sub-scales observing, describing, acting, and accepting.

The Psychopathic Personality Inventory (PPI) was calculated by summing the sub-scores of the Machiavellian Egocentricity, Social Potency, Cold-heartedness, Carefree Non-planfulness, Fearlessness, Blame Externalization, Impulsive Nonconformity, and Stress Immunity and reverse scoring according to standard procedure.

The Spiritual Meaning Scale (SMS) score was calculated by summation of the individual questions.

Lastly, reported social exclusion was measured through the Cyberball questionnaire. For scoring, the Cyberball assessment was broken into two categories. The first categories was "Invisibility", the question that related to exclusion from the game (*How much did you feel "invisible" during the online game?*). The second category was "Four Needs", a summation of all of the questions.

To evaluate the effect of the Cyberball task on emotional and physiological stress responses between the experimental and control group in session 1, the difference between the baseline score and the score after game 2 (the conditioned round of Cyberball) was of most interest. Delta values were calculated for RSA, THM, POMS, and cortisol between baseline and the conclusion of the second game (see Table 1). To analyze differences between the control and experimental groups, the resulting delta values were compared using independent t-tests.

Psychological assessments of the experimental and control group are shown in Table 2. To evaluate the effect of individual differences in dispositional mindfulness, personality and current depressive and anxiety symptom status, standard correlation values were calculated using two-tailed bi-variate correlations between the RSA and THM delta values for session 1, the SMS, the KIMS, the PPI, DASS-42, and the Cyberball assessment.

## Results

## **Results Session 1**

#### **Physiological Stress Response Results**

Of the original 10 subjects in the excluded group, cortisol was successfully collected and analyzed from 9 subjects the first session and 9 the second session. Cortisol was successfully collected for all 8 subjects in the control group. Data from the excluded group was lost due to due to cracking in the test tubes. Mean delta values and standard deviation are presented in Table 1. There was no significant difference between the delta values of the control group versus the excluded group for cortisol levels (t = 1.035, p = 0.317). There was a trend in the data towards a decrease in cortisol levels in both the experimental and control group (see Figure 6).

Heart Rate data was collected from 8 subjects in the first session from the ostracized group, 7 from the second session, and 7 from the control group. Data was lost in four cases due to 2 Biologs not being properly prepared and booted, and electrode stickers falling off prematurely in 2 cases. Mean delta values and standard deviation for THM and RSA are presented in Table 1. There was no significant difference between the THM delta mean values of the control group versus the excluded group from baseline to time 2 (t = -0.425, p = 0.678). There was no significant difference between the delta values of the control group versus the excluded group for RSA values (t=1.761, p = 0.104).

There was a positive delta in RSA. The positive delta signifies an increase in RSA from *baseline* to *game 2* points. Based on current conceptualizations of HRV, this would suggest that the rise in RSA represented a reduction in stress. The expected trend for a stressor would be a

decline in RSA during and immediately after experience with the stressor. If a significant stressor was present, the mean RSA through *game 2* would have been significantly lower than the mean for both the *baseline* data and *game 1* (see Figure 7 and Figure 8 for visual representation of RSA and THM trends). The trend followed by TMH, in response to stress, are less obvious than RSA. In the excluded group there was an almost significant correlation between RSA and THM (r = .715).

#### Affective Results

The psychological assessments, including the POMS, KIMS, SMS, PPI, and DASS-42, were collected for 17 subjects, 10 from the ostracized group and 7 from the control group. One subject's data was removed from the control group due to misunderstanding of the directions and marking arbitrary answers.

Prior to analysis, all of the POMS raw scores were transformed to positive values by adding a coefficient of 20. The transformed mean score of the change in POMS from *baseline* to *time 1* (after Cyberball) is shown in Table 1. There was not a significant difference between the control and excluded groups' change in POMS scores (t = 1.960, p = 0.160). The negative mean POMS delta values in both the excluded and control groups from *baseline* to *time 1* indicates an increase in positive mood throughout the study. The larger mean POMS delta value of the control group suggests that the mood state of the excluded group improved at a lower rate than the control group. However, both groups moved in the same direction towards improvement in mood. This trend was present in the experimental group in session 2 with the experimental group. From *POMS baseline* to *POMS 1* there was a decline and from *POMS 1* to *POMS 2* there was an incline. The trends in POMS are visually represented in Figure 3. Due to the unexpected

results, we examined each subject's POM trendline individually. The majority of participants followed the average trend. Trends of the individual control subjects are visually represented in Figure 4. Trends of the individual excluded subjects are visually represented in Figure 5.

#### **Psychological** Assessments

The mean values and standard deviations for the other psychological assessments are displayed in Table 2. The excluded group had higher mean values in their baseline POMS, DASS-42, KIMS, and SMS. The control group had a higher mean PPI score. There was a negative significant correlation between the PPI and Stress (sub-scale of DASS) of r = -.502 and a negative correlation between KIMS and PPI of r = -.659. other associations were insignificant. Non-significant correlations include a positive trend between POMS and DASS (and its subscales), positive correlation between KIMS and DASS (and its subscales), and a negative correlation between SMS and DASS, KIMS, and PPI.

Within the excluded group, there were no significant correlations between RSA, THM, POMS, and cortisol (see Table 3) or with the invisibility score (Table 4). Furthermore, within the same group there were no correlations between RSA, cortisol, POMS, and the psychological assessments (DASS, KIMS, SMS, and PPI) (Table 4). The Delta THM score was strongly correlated to the DASS score (R = .845) and it's subscales of anxiety (R = .864) and depression (R = .792) (Table 4).

The "Four Needs" (based on the four needs criterion) and "Total Exclusion" (a sub-score) mean scores are listed in Table 1. Between the control and excluded groups, the "Four Needs" calculation was not significant (t = 1.430, p = 0.173). However, the difference between the

"Invisibility" scores was significant (t = 2.628, p =0.019) between the excluded and control groups.

## **Results Session 2**

To test the hypothesis regarding the repeatability of Cyberball within the experimental group, the delta values of the POMS, RSA, THM, and cortisol of session 1 and session 2 were compared using paired t-tests. None of the resultants comparing session 1 to session 2 were significant. Delta THM (t = =.811, p = .448), Delta RSA (t = -0.843, p = 0.431), Delta POMS (t =-0.243, p=0.814) and delta cortisol (t= 1.183, .p=0.271). The trends followed the same pattern for most measures. All of the POMS scores for session 2 were lower than POMS scores for session 1 (Figure 10); however, the same trend is followed. RSA means also follow the same trendline (Figure 7). THM mean trends between session 1 and session 2 had more variance (Figure 8). Cortisol mean trends were very consistent between session 1 and session 2 (Figure 11).

#### **Discussion**

Study Limitations

The present study is limited in statistical power due to the small sample population. Thus, we suspect that at least a portion of our failure to detect statistically significant differences may be the result of type II (β) error. Unfortunately, *a priori* determination of requisite sample size by study power was not possible, as previous studies analyzing heart rate variability and salivary cortisol levels in this model system have not been reported. "Stress" is a highly individualized response, dependent on a multitude of factors. Perceived and experienced stress varies for each individual, depending on complex biological factors<sup>49</sup>, different personality characteristics<sup>50</sup>, and past experiences<sup>51</sup>. Therefore, our study may have been confounded by our inability to identify appropriate inclusion criteria and analysis parameters. Finally, in addition to sample size and detection methodology, one may also consider the relatively innocuous nature of the Cyberball task compared to other more robust stressors. A more powerful stressor and subsequently more pronounced stress responses in study participants would be helpful in delineating potential differences among experimental groups.

In terms of other limitations, it is prudent to consider more careful control of the myriad external factors that may have compromised the study. For example, allowing participants more time to acclimate in the new setting prior to performing the experimental task may have permitted better normalization of baseline values in the assessed parameters, and may have reduced baseline differences between the experimental and control group. Given the importance of routine sleep-wake cycles in modulating cortisol production, regular sleep schedules were recommended to the participants; however, it is unclear if the subjects adhered to these suggestions. Furthermore, dietary patterns, especially alcohol consumption, were not controlled or assessed and may have contributed to outlying data points.

Other limitations of this study include small group size, the strength of parameters used to measure stress, and potential irregularities in physiological data. Because stress is such an individual response, in any stress model, it is crucial to have a large enough subject size reacting to the stress as well as tools sensitive enough to be able to detect subtle responses of stress. A potential shortcoming in this study was the parameters used. For example, psychological assessments that measure mood are rarely used in laboratory social exclusion stressors. In fact, it has been theorized that broad mood measures, like the Positive and Negative Affect Schedule (PANAS)<sup>52</sup> and Profile of Mood States (POMS), are insensitive to short phases of rejection or acceptance.<sup>53</sup> Our data is consistent with these findings. The presents the issue that the POMS might not have been sensitive enough to detect stress inflicted by Cyberball. Further, it is possible that social stressors like Cyberball evoke stress so subtle it's not significant in the current study due to the parameters used or the number of subjects

### Study Implications

The reduction in the mean POMS, RSA, THM, and cortisol values suggests a decline in psycho-physiological stress experienced by both the control and experimental group from baseline to completion of the Cyberball task. However, correlation analyses failed to identify statistically significant interdependence between the assessed random variables (POMS, RSA, and cortisol). Given these inconsistencies and failure to demonstrate correlation, meaningful interpretations of experiments utilizing Cyberball as a model of social exclusion to elicit stress responses are precluded. Alternatively, these experiments do highlight important considerations regarding the methodology and design of similar studies.

Based on the results described here, we conclude that Cyberball is not effectively powerful in eliciting a substantial and consistent psycho-physiological stress responses across a diverse pool of subjects. Similar to other reports from studies utilizing Cyberball, distress, identified by feelings of invisibility, was reported in the excluded group. "Invisibility", in this study, was the only significant difference between the control (included) and experimental group (excluded). Although this finding clearly validates the ability of Cyberball to provoke feelings associated with social exclusion (invisibility), the absence of well-recognized physiological determinants of stress suggests that the model is incomplete and thus critically limited in its capacity to accurately recapitulate stress responses due to social exclusion.

As mentioned previously, an adjunctive focus of this study was to explore specific personality traits under conditions of stress. To this end, we administered a range of psychological assessments measuring depression, anxiety, mindfulness, psychopathy, and spiritual meaning. Within the group that underwent social exclusion in Cyberball, in a bi-variate two-tailed correlation analysis, there were no significant correlations between the physiological (RSA and cortisol), psychological (POMS), and distress (invisibility score) and the scales of mindfulness, spiritual meaning, psychopathy, and depression and anxiety. These findings suggest that particular differences in mindfulness and spirituality did not influence the levels of stress within this subject group.

Depression and anxiety values taken from the Depression Anxiety Stress Scale were inversely correlated to the Psychopathic Personality Inventory values, which is consistent with previous studies.<sup>54</sup> Positive correlations were identified between both the Kentucky Inventory of Mindfulness Skills (KIMS) and Spiritual Meaning Scale (SMS) and between the depression and anxiety values (DASS). The literature addressing spirituality and its relationship to mental health is complicated by obvious discrepancies and overtly opposite conclusions. Some investigators report that high spiritual meaning and mindfulness are personality characteristics associated with lower levels of depression and anxiety, inconsistent with our findings.<sup>55</sup> On the other hand, others have found religiosity to be oppositely correlated with mental health.<sup>5657</sup> The effects of religiosity (a broad term including components of spiritual meaning and mindfulness) on the state of mental health are related to a number of complex factors not examined in this study such as race<sup>58</sup>, nationality, and socioeconomic status. Thus, the impact of these more subtle externalities remains unappreciated in models of stress responses to social exclusion and future study designs will benefit from incorporation of these considerations.

Our hypothesis that Cyberball could be utilized repeatedly to trigger psychophysiological stress responses is not supported by the results obtained in the present study. We attribute this finding to the fact that Cyberball is not a strong enough stressor to consistently elicit psycho-physiological stress responses. However, our results indicated that one stress response parameter, "invisibility", could be generated consistently and reliably using the Cyberball task.

Subjects in the excluded group reported feelings of invisibility upon the second completion of Cyberball, indicating the task may be useful in other types of longitudinal studies. Additionally, similar values in POMS, HRV and cortisol were observed in subjects following both iterations of the Cyberball task and the variability between these data points was statistically insignificant. Further evaluation of these parameters with a greater magnitude variance as well as longer latency periods between iterations will yield valuable insight into the utility of Cyberball as robust model of psycho-physiological stress.

Careful scrutiny of the present body of literature regarding Cyberball based experimental designs found little direct evidence to support the use of Cyberball as a psycho-physiological stressor. fMRI studies have provided some indirect support for minor neuro-physiological changes associated with participation in the Cyberball task. However, the subjects' momentary moods were not reported in these studies and the study design omitted the critical control of a separate "included" group. In light of the findings we report here, we believe that there remain underappreciated issues regarding the experimental modeling of psycho-social stress that should be revisited before broader application of this methodology is admissible to the larger scientific community.

In conclusion, it cannot be determined if Cyberball elicits psychological and/or physiological reactions of stress based on our study. We observed no statistically significant changes in the parameters used to assess psycho-physiological distress. Previous studies using Cyberball based experiments relied heavily on post-hoc analysis through a self-reported survey based on the "Four needs criterion". Such surveys have been shown to introduce bias by cuing participants as to expected answers. It is clear from our extensive literature analysis and the shortcomings of the present study that accurate experimental modeling of stress responses incited by Cyberball is not a foregone conclusion. The caveats and pitfalls experienced in our pilot study serve to elucidate critical design flaws to be addressed in future studies. In summary, be examined with the utmost objectivity and scientific integrity if meaningful and valid discoveries are to be realized.

# Table 1: Mean Delta values from baseline to post-Cyberball (Excluded group is condition 1 and

*control is condition 2)* 

Variable	Condition	N	Moon	Std.	Std. Mean	4	d f	
Variable Cortisol Pre			Mean	Deviation	Error	t	d.f.	р
Corusoi Pre	1	9	.2668	.22359	.07453	-1.146	15	.270
	2	8	.4245	.33871	.11975	-1.118	11.909	.286
Cortisol Post	1	9	.1604	.09312	.03104	929	15	.368
	2	8	.2221	.17355	.06136	897	10.442	.390
Delta Cortisol	1	9	1063	.13343	.04448	1.035	15	.317
	2	8	2024	.24042	.08500	1.001	10.659	.339
DeltaPOMS	1	10	-1.6000	8.07190	2.55256	1.455	15	.166
(Time 2 - Time 1)	2	7	-6.4286	3.95209	1.49375	1.633	13.793	.125
DeltaPOMS	1	10	2.1000	7.15619	2.26299	397	15	.697
(Time 3 - Time 1)	2	7	3.4286	6.18755	2.33867	408	14.198	.689
POMS1	1	10	27.3000	19.24145	6.08468	1.010	15	.329
	2	7	19.2857	9.63871	3.64309	1.130	13.925	.278
POMS2	1	10	25.7000	14.94471	4.72593	2.038	15	.060
	2	7	12.8571	8.59125	3.24719	2.240	14.617	.041
POMS3	1	10	29.4000	16.64131	5.26245	1.013	15	.327
	2	7	22.7143	5.76525	2.17906	1.174	11.829	.264
FourNeeds	1	10	16.2000	4.34102	1.37275	1.430	15	.173
	2	7	12.7143	5.73627	2.16811	1.358	10.635	.202
Invisibility	1	10	5.6000	1.77639	.56174	2.628	15	.019
	2	7	2.8571	2.54484	.96186	2.462	10.014	.034
THM1deltaB1	1	8	.5028	.70642	.24976	1.960	13	.072
	2	7	1132	.46555	.17596	2.016	12.174	.066
THM1deltaB2	1	7	.3788	1.01760	.38462	425	12	.678
	2	7	.5667	.57465	.21720	425	9.473	.680
RSA1deltaB1	1	8	0573	.58187	.20572	2.813	13	.015
	2	7	7810	.37468	.14162	2.897	12.049	.013
RSA1deltaB2	1	7	.0357	.52450	.19824	1.761	12	.104
	2	7	4096	.41549	.15704	1.761	11.403	.105

Variable	Condition	Ν	Mean	Std. Deviation	Std. Error Mean
DASS	1	10	22.7000	18.09880	5.72334
	2	7	8.7143	3.94606	1.49147
KIMS	1	10	114.6000	8.90942	2.81741
	2	7	105.5714	23.20099	8.76915
PPI	1	10	121.9000	8.51730	2.69341
	2	7	133.2857	12.69796	4.79938
SMS	1	10	62.1000	11.40614	3.60694
	2	7	53.1429	9.68553	3.66079
Depression	1	10	5.3000	6.25478	1.97793
	2	7	1.7143	2.81154	1.06266
Anxiety	1	10	6.7000	5.12185	1.61967
	2	7	2.0000	1.15470	.43644
Stress	1	10	10.7000	8.23340	2.60363
	2	7	5.0000	2.00000	.75593

**Table 2: Descriptive Statistics**: Mean psychological assessments between groups (Excluded group is condition 1 and control is condition 2)

## Table 3: Excluded Group (Session 1): Stress Variable Correlation Values

Variable		THM	RSA	POMS	Cortisol
THM	Pearson Correlation	1	.170	508	713
	Sig. (2-tailed)		.715	.245	.112
	Ν	7	7	7	6
RSA	Pearson Correlation	.170	1	.135	265
	Sig. (2-tailed)	.715		.773	.612
	Ν	7	7	7	6
POMS	Pearson Correlation	508	.135	1	296
	Sig. (2-tailed)	.245	.773		.440
	Ν	7	7	10	9
Cortisol	Pearson Correlation	713	265	296	1
	Sig. (2-tailed)	.112	.612	.440	
	Ν	6	6	9	9

Scale		Invisibility	FourNeeds	THM	RSA	POMS	Cortisol
SMS	Pearson Correlation	.123	357	253	043	007	.028
	Sig. (2-tailed)	.735	.311	.585	.926	.986	.943
	Ν	10	10	7	7	10	9
DASS	Pearson Correlation	353	.455	.845*	146	457	248
	Sig. (2-tailed)	.317	.187	.017	.755	.184	.520
	Ν	10	10	7	7	10	9
KIMS	Pearson Correlation	018	.359	.637	284	599	129
	Sig. (2-tailed)	.960	.309	.124	.537	.068	.741
	Ν	10	10	7	7	10	9
PPI	Pearson Correlation	370	378	065	.526	.529	106
	Sig. (2-tailed)	.292	.281	.890	.226	.116	.786
	Ν	10	10	7	7	10	9
Depression	Pearson Correlation	488	.325	.717	068	300	375
	Sig. (2-tailed)	.152	.360	.070	.885	.400	.321
	Ν	10	10	7	7	10	9
Anxiety	Pearson Correlation	491	.313	.864*	017	341	350
	Sig. (2-tailed)	.150	.379	.012	.971	.335	.355
	Ν	10	10	7	7	10	9
Stress	Pearson Correlation	100	.558	.792*	266	565	056
	Sig. (2-tailed)	.783	.093	.034	.564	.089	.885
	Ν	10	10	7	7	10	9

 Table 4: Excluded Group (Session 1): Stress Variables and Psychological Assessment Correlation Values



## Figure 3: Mean POMS score by time (values transformed)

Figure 4: POMS score trends by individual experimental subjects (session





Figure 5: POMS score trends by individual control subjects









Figure 8: THM score trends by mean





Figure 9: Session 2 and Session 1 POMS mean (values transformed)





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