

Methodological and Statistical Challenges in the Study of How Anxiety and Stress Impair Working Memory Performance

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Discipline

Psychology [D3]

Sub-discipline

Clinical and Abnormal Psychology [SD-Psych-3]

Academic Level

Advanced Undergraduate

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Kim Pulvers, PhD, MPH, is a clinical health psychologist and associate professor at California State University San Marcos. Dr. Pulvers received her PhD in Clinical Health Psychology and Master of Public Health from the University of Kansas and completed her postdoctoral fellowship at the University of California, San Diego. Dr. Pulvers' research focuses on stress and health. Her areas of interest include health disparities, nicotine and tobacco use and other addictive behaviors, mental health, and positive psychology.

Published Articles

Hood, A., Pulvers, K., & Spady, T. J. (2013). Timing and gender determine if acute pain impairs working memory performance. *The Journal of Pain, 14*, 1320-1329.

Hood, A., Pulvers, K., Spady, T. J., Kliebenstein, A., & Bachand, J. (2015). Anxiety mediates the effect of acute stress on working memory performance when cortisol levels are high: A moderated mediation analysis. *Anxiety, Stress, & Coping, 28*, 545-562.

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Abstract

This case study presents an overview of the major challenges and successes encountered assessing whether working memory performance was impaired in healthy individuals with high levels of anxiety when their cortisol levels increased after acute stress. The study presented used an experimental design and quantitative data analysis using a moderated mediation model. In the case study that follows, we describe anxiety disorders, stress and the resulting physiological responses, and how anxiety and stress can impair working memory performance. In addition, we reflect on the use of healthy college students in research that has clinical implications, how to design a study that uses an experiment stressor, and the potential pitfalls of salivary cortisol collection and analysis. We also discuss the changes we would implement if we were to conduct similar research in the future. This case study highlights the use of an experimental design and statistical modeling to answer interesting questions about human behavior.

Learning Outcomes

By the end of this case, students should be able to

- Understand how anxiety disorders and stress can influence cognition, specifically working memory performance
- How to design an experimental study in which healthy participants are randomly assigned to groups
- To understand how to conduct statistical analyses of conditional indirect effects, commonly known as a moderated mediation model

Case Study

Project Overview and Context

Anxiety is an emotion characterized by feelings of worry and tension with accompanying anticipation and preparation for future harm. Excessive anxiety can be maladaptive; however, some anxiety is useful and adaptive and serves as a warning of potential danger (Spielberger, 2013). For example, when we experience a stressor, whether from internal worry or external threat, our bodies trigger something called the “fight-or-flight” response. The fight (using aggression in response to a threat) or flight (fleeing in response to a threat) response was discovered in 1932 by physiologist Walter Cannon and represents a genetic system designed to protect us from harm. The fight-or-flight response produces physical symptoms driven by the sympathetic nervous system/adrenergic response. These physical symptoms include the following:

- Sweating;
- Stomach and chest pain or discomfort;
- Heart palpitations (pounding or racing heartbeat);
- Shortness of breath;
- Feelings of choking;
- Numbness or tingling;
- Trembling or shaking;
- Dizziness;
- Shivering;
- Feeling extremely hot or cold.

In the modern world, fighting a perceived threat is mostly inappropriate and we often cannot physically flee. Thus, individuals experiencing high levels of anxiety must stay and take the difficult test, or complete the presentation in front of colleagues, or remain in a crowded

room full of strangers. In these circumstances, the fight-or-flight response is triggered without resolution, causing individuals to have feelings of aggression and hypervigilance and become over-reactive in both threatening and non-threatening situations. Individuals with high levels of anxiety will subsequently begin to avoid feared situations. At first, they will experience feelings of relief and lowered anxiety. Relief, however, is short-lived. Unfortunately, this avoidant behavior is extremely reinforcing and so it becomes harder and harder for the individual to face the dreaded situation.

Feelings of anxiety can span the spectrum from low levels, such as anticipation of a night out with friends, to debilitating levels, such as individuals experiencing such significant fear that they don't leave their homes. Individual who experience high levels of anxiety are not considered to be suffering from a psychological disorder unless they report feelings of distress about their symptoms or experience significant impairment in their life (e.g., problems at school or work due to avoiding situations which trigger anxiety). There are different types of anxiety disorders that reflect feeling distress or impairment from different symptoms. These anxiety disorders are as follows:

- Separation anxiety disorder;
- Selective mutism;
- Specific phobia;
- Social anxiety disorder;
- Panic disorder;
- Agoraphobia;
- Generalized anxiety disorder;
- Substance/medication-induced anxiety disorder;

- Anxiety due to a medical condition;
- Unspecified anxiety disorder.

The fight-or-flight response is supported by the neuroendocrine system which prepares the body for action through a brain system called the hypothalamic–pituitary–adrenal (HPA) axis (Tsigos & Chrousos, 2002). As part of a negative feedback loop, the hypothalamus releases corticotropin-releasing factor (CRF). CRF binds to receptors on the pituitary gland and adrenocorticotrophic hormone (ACTH) is released. ACTH then binds to receptors on the adrenal cortex and stimulates the release of cortisol, a steroid hormone. This process continues until cortisol reaches the levels that the body needs to respond to stressors, then cortisol released by the adrenals inhibits the hypothalamus and the pituitary gland (so they stop sending signals to produce more cortisol) thus exerting a negative feedback. At this point, systemic homeostasis returns.

The physiological response from stress and anxiety can disrupt the ability to concentrate. Mental processes that may be particularly vulnerable to anxiety and stress are executive functions. Executive functions enable us to plan, inhibit responses, focus attention, and juggle multiple tasks successfully (Miyake, Friedman, Rettinger, Shah, & Hegarty, 2001). For example, an air traffic control system must manage the arrival and departure of planes on multiple runways and prioritize tasks to achieve goals while filtering out any distractions. The human brain works in a similar way. Executive functions appear particularly vulnerable to disruption due to high levels of anxiety is working memory. Working memory is an executive function with a limited capacity that is responsible for temporarily holding information available for processing (Baddeley, 2012). Anxiety may influence working memory performance by disrupting functions involved in inhibition and switching during task performance.

The project described in this case study investigated the relationship between anxiety, stress, and the executive function, working memory. Specifically, we wanted to know whether stress impaired working memory performance. Importantly, we were interested in whether individuals with higher levels of anxiety had impaired working memory performance after exposure to acute stress. In sum, we were interested in whether the physiological response to stress (cortisol release) influenced whether individuals with high levels of anxiety had impaired working memory performance.

Research Design and Method

In designing this study, we spent a long time selecting methods that we could use to test our question. For many months prior to the study, we considered and pilot tested tasks and materials that would not only help answer our questions but were also practical. One vital decision during this process was to video-record the testing session. This allowed us to have independent research assistants monitor the testing for standardization in administration.

Before beginning the study, the research team discussed and tested the optimal way to have participants experience acute stress. Other studies in the lab had used a cold pressor task, which was well validated in the literature. A cold pressor task generally has participants submerge their non-dominant hand up to the wrist for as long as tolerable in a refrigerated bath that continually circulated 0°C water for an uninformed maximum duration. In the case study described here, we wanted the participant's hands to be free to complete a computerized working memory test. As can often happen in research, we ended up using a working memory test that required verbal but not physical responses, but by this time, we had devised a forehead cold pressor task that was like tasks that had been used in other research studies. We also researched

to find a working memory test that did not add significant additional acute stress. For example, we did not use the Paced Auditory Serial Addition Test (PASAT), as it has been used in previous research as a stressor.

Participants were college students who participated for course credit, which can result in a homogeneous sample regarding age, ethnicity, and socioeconomic status. Luckily, our convenience sample was largely heterogeneous, although the sample skewed young, which is expected from a college sample.

To make sure participants could tolerate the cold pressor task, we excluded participants if they had circulatory problems (e.g., Raynaud's disease), peripheral neuropathy, thyroid problems, diabetes, lupus, other connective tissue disorders, cardiovascular disorders, high blood pressure, and/or hypertension. Other exclusion criteria were if they were taking any pain or psychotropic medications, as we wanted to see the influence of acute stress and anxiety without the influence of medications. Participants could also not have a history of fainting or seizures, significant trauma or history of pain disorders, significant weight loss or major surgery within the past 6 months, substance abuse, a neurological condition, or be pregnant. Our exclusion criteria were determined through self-report of participants; thus, it is possible that our sample included some individuals who had excluded disorders or problems but did not disclose to them research assistants.

To accurately measure cortisol release, participants could not eat or drink anything but water for 1 hr before the study. These restrictions resulted in 18 participants being excluded from the study, which was not an insignificant number. Importantly, only one participant could not tolerate the cold pressor, which indicated that we had a task that induced stress but was not

unbearable for participants. To attempt to control for cortisol diurnal variation, we tested participants between 11 a.m. and 3 p.m., as cortisol levels are most stable during this period.

We randomly assigned participants to either a control (no acute stress) or experimental condition (experience acute stress) using an online random number generator. Random assignment ensured that the groups were like each other (i.e., equivalent) prior to the testing. To maintain as much parity between groups as possible, participants in the control condition wore the forehead pad attached to the cold pressor, but the pad did not contain freezing cold water. In addition, at the beginning of the study, all participants were told that they may or may not experience discomfort, although participants did not have prior knowledge about condition assignment.

Materials Used

After much discussion and time, we settled on the following measurements of anxiety, stress, cortisol, and working memory performance.

Anxiety

Participants completed the Beck Anxiety Inventory (BAI; Beck, Epstein, Brown, & Steer, 1988) as our measure of anxiety prior to acute stress. Participants completed the BAI after our baseline cortisol measurement in case completing the measure induced a stress response. The BAI is 21-item self-report paper and pencil inventory and is widely used in research and clinical settings. Higher scores represent higher levels of anxiety. The BAI focuses on somatic (e.g., heart racing, dizziness) symptoms of anxiety and was developed as a measure to discriminate between anxiety and depression. Although the BAI minimizes overlap with depressive symptoms, the physical symptoms of anxiety assessed can overlap with some physical aspects of medical conditions.

Stress

In our cold pressor task, participants had a pad attached to their forehead with cold water distributed throughout the entire surface. The temperature of the cold water was regulated at 0°C.

Cortisol

We used saliva samples for cortisol measurement. We chose this method as it was non-invasive and could be completed in a psychology lab by research assistants. Saliva samples had also been used extensively in previous literature. Participants chewed on a waxed sheet (Parafilm[®]) to stimulate saliva and then passively salivated for 1 min through a short straw into a Cryovial tube. Some participants did have trouble with initial saliva production, and we were not able to get samples from all participants. After collection, researchers capped, labeled, and froze samples at -20°C in a non-self-defrosting freezer. This step was important to maintain the integrity of the samples for analysis.

Working Memory

We used a letter–number sequencing test as our measure of working memory. In this test, the research assistant verbally presented different sets of increasingly longer sequences of intermixed letters and numbers at a rate of one per second. After each sequence, participants repeated the numbers in numerical order and letters in alphabetical order. The test consists of 21 trials with sequences that range from two stimuli (e.g., B-4) up to a maximum length of eight stimuli. The research assistant presented three trials at each length and discontinued the test when the participants failed on three consecutive trials of the same length. We tested working memory performance during and after acute stress. To minimize the possibility for practice effects, we administered alternate forms of the test. This working memory test was chosen as it

was difficult enough to test limits but minimized frustration. It could also be completed in less than 5 min, which supported safety guidelines for exposure to the forehead cold pressor task.

The study procedure is described as follows:

1. Random assignment to control or experimental group;
2. Completion of questionnaire to determine demographics and exclusion criteria;
3. Baseline saliva cortisol collection;
4. Completion of the measure of anxiety (BAI);
5. Exposure to cold pressor task (without cold water in the control condition) and first working memory task simultaneously;
6. Break to allow cortisol release as a response to the cold pressor (participants read magazines);
7. Completion of the second working memory test;
8. After testing saliva cortisol collection;
9. Debriefing.

One unanticipated result of the study design was that when we later analyzed cortisol, we found that there was an increase in both the control and experimental conditions. It appears that either being told that they may experience stress or completing the working memory test may have induced a stress response. It is also possible that there was another unknown reason. The cortisol response was higher in the experimental condition, as would be expected, but the fact that participants in the control condition (no acute stress) had a physiological stress response indicates the many ways in which stress can occur, and in the future, we would aim to find a research design that reduced stress in the control condition.

Other challenges for this study were as follows:

- Making sure the cold pressor task induced stress but was not unbearable for participants;
- Reminding and monitoring that participants did not eat or drink in the 1 hr prior to beginning the study;
- Handling medical disorder/illness disclosures from participants in a sensitive way;
- Making sure the cold pressor remained in the 0°C range throughout testing;
- Making sure that the working memory test was administered in the same standardized way by all research assistants.

Statistical Design and Analysis

In this study, the statistical design was almost as important as the research design. A moderator is a variable that affects the *strength* of the relationship between the predictor (independent variable [IV]) and criterion (dependent variable [DV]). Mediation *explains* or *causes* the relationship between the IV and DV (Baron & Kenny, 1986). Our model combined moderation and mediation as we wanted to determine not only group differences but also change in the IV (experimental vs control condition) and the DV (working memory) only in the presence of another anxiety (mediation) and only at certain cortisol levels (moderation). Thus, this design required a moderated mediation analysis.

Figure 1 shows the conceptual moderated mediation model used in the project, in which cortisol levels influence working memory through the combination of condition (stress or control) and anxiety.

Figure 1.

Caption: Conceptual moderated mediation model.

To test the relationships between condition (stress vs control), anxiety, cortisol, and working memory performance, we used the PROCESS procedure for SPSS (Hayes, 2012). SPSS is a commonly used statistical software package. PROCESS is an easy add-on package that produces conditional indirect effects in the moderated mediation model. In Figure 1, the indirect effect is the product of path coefficients “A” and “B.” To test for indirect effects, PROCESS utilizes bootstrapping, which is a non-parametric resampling statistical procedure. Because the sampling distribution of the statistic in bootstrapping is formulated through resamples from the data set, there are no assumptions based on normality theory, it avoids power problems associated with non-normally distributed variables, and can be applied to small samples with more confidence. Bootstrapping is remarkably simple to implement, but it would not be feasible without modern computing power. Bootstrapping performs computations on data to estimate statistics that are then computed from the same data, for example, the data are “pulling itself up by its own bootstrap,” which essentially means that the data use their own resources (resamples) to create the sampling distribution (Efron & Tibshirani, 1986).

To gain the confidence and knowledge to run these analyses, we collaborated with a graduate-level statistics professor and attended statistical workshops. In addition, the authors of PROCESS posted detailed descriptions online and were available for questions via online message boards. PROCESS produced asymmetric bias corrected and accelerated 95% confidence intervals (CIs) to test for significance. Bias-corrected and accelerated 95% CIs adjust for any bias and skewness in the bootstrapped distribution. Thus, 95% CIs produce a more reliable estimate. If zero was not within the 95% CI, we concluded that the indirect effect was significantly different from zero at $p < .05$, two-tailed. For our study, a significant indirect effect meant that anxiety mediated the relationship between condition (IV) and working memory (DV).

Although determining whether our model was significant is important, we also wanted a measure of effect size so we could see the size of the difference. We used kappa-squared as the measure of effect size in mediation analyses because it is standardized and insensitive to sample size. Kappa-squared represents the proportion of the total possible effect in the sample and it can be interpreted analogous to r^2 (a commonly used measure of effect size in correlations), with a kappa-squared of .01, .09, and .25 representing small, medium, and large effects, respectively.

Moderated mediation emphasizes the estimation of the extent of Indirect effect of an IV (X) on the DV (Y) through the mediator (M) depending on the moderator (W).

Thus, we tested

Indirect effect of stress vs control (X) on working memory (Y) through the mediator, anxiety (M) depending on the moderator, cortisol (W) (see Figure 1).

Practical Lessons Learned

One of biggest lessons we learned through this project involved the collection and analysis of the cortisol samples. We collected cortisol to determine whether increased cortisol following acute stress resulted in worse working memory performance. Below is a list of some of the challenges related to *cortisol values*, that if we conducted this research in the future, we might change or modify:

1. It would have been beneficial to have salivary samples at multiple time points to give a clearer picture of the moderating effect of cortisol. If money and time had not been an

issue, we would have collected cortisol at 15- to 30-min intervals after acute stress.

Measuring cortisol over a longer period would have allowed us to see when the body returned to homeostasis after the HPA stress response.

2. Cortisol, like many other physiological processes in the body, has a circadian rhythm.

Normal individuals, with a normal HPA axis response have very low cortisol levels at around midnight. Cortisol levels build up overnight, peak in the morning, and then decline slowly throughout the day. Because of the diurnal variation of cortisol, we were concerned that if we tested early in the morning, cortisol would naturally be higher than if we tested during the afternoon. This might mean that we would observe poorer working memory performance for individuals tested in the morning, because of the cumulative effects of naturally high cortisol and acute stress, and observe better working memory performance for individuals tested in the evening, because of naturally low cortisol. As noted above, we attempted to control for cortisol diurnal variation, we tested participants between 11 a.m. and 3 p.m., as cortisol levels are most stable during this period. We did not find a significant difference between the baseline cortisol levels of our participants, which provides support for our findings. However, it would have been advantageous to test participants over a shorter period, for example, 11 a.m. to 1 p.m., or all at the same time, for example, 11 a.m., to further control for the diurnal variation of cortisol. For this study, reducing the availability of testing sessions was not possible as data collection was incredibly slow and we were limited by the availability of research assistants. It would also have been interesting to test participants over multiple time points to determine whether the timing of cortisol collection was important in the magnitude of working memory impairments.

3. When beginning this study, we had the opportunity to collaborate with a biology lab in the analysis of our cortisol samples. Through this collaboration, we learnt the difficulties and limitations of using immunoassay kits designed and validated for the quantitative measurement of salivary cortisol. Before the study, both hands-on and didactic training were completed over many weeks. Once we began cortisol data analysis, we found that variation both within (same plate) and between (different plate) assays was not always <15% (the accepted standard). The goal is to have as little variation as possible, but there will always be some variation; as such, a standard is necessary so that data can be compared across studies. Because we had some variation >15%, we retested some assays. It was at this point that it became clear that larger quantities of saliva from each participant would have been useful. For some participants, we were not able to achieve variation of <15%, as we had small quantities of saliva. Therefore, we could not retest their sample and these participants' cortisol values were not available for analyses.
4. Cortisol is released as part of the fight-or-flight response. Other neurochemical changes not tested in this study, such as the release of catecholamines, could also have influenced working memory performance. Catecholamines are hormones mainly produced by the adrenal glands and include dopamine, norepinephrine, and epinephrine. The reason we did not analyze these catecholamines was also because of cost and an insufficient amount of saliva collected from participants. They could also have proven to be moderators, but we made the decision to prioritize our resources and only analyze cortisol.

Other lessons we learned through this project involved the measurement of anxiety in our participants. Below is a list of some of the challenges related to *anxiety*, that if we conducted this research in the future, we would change or modify:

1. Ideally, this research would have included a clinical population, for example, individuals diagnosed with an anxiety disorder, determined through assessment and clinical interview. In addition, there would have been a control group of individuals without an anxiety disorder. Time and monetary constraints made this impractical; instead, participants were a convenience sample of university students receiving college credit. We found support for our moderated mediation model even in university students with anxiety below clinically meaningful levels. It is unclear whether participants with moderate–severe anxiety would have the same physiological response, as their cortisol may remain chronically high with less variation. We also do not know whether individuals with different anxiety disorders, for example, social anxiety disorder or generalized anxiety disorder, have the same response and resulting working memory impairments.
2. We used the BAI as our measure of anxiety. The BAI can be thought of as a measure of “prolonged state anxiety.” The BAI was designed to measure clinical anxiety while minimizing the overlap between depression and anxiety. It might be useful to use a different measure of anxiety in this project. For example, we could have used a measure of trait anxiety to determine anxiety participants’ usual anxiety levels.
3. If we were to use the BAI in future research, we would test anxiety before and after stress, for example, at the same time as cortisol collection. This would have provided more information about how anxiety changes due to acute stress and cortisol release.

Conclusion

The project described in this case study found working memory impairments for healthy individuals with high levels of anxiety only when cortisol levels are high (Hood, Pulvers, Spady, Kliebenstein, & Bachand, 2015). This project provided new information into the underlying physiological mechanisms at play during stress. Although there were limitations, the findings have interesting clinical, professional, and educational implications.

This project allowed us to see the feasibility of using a forehead cold pressor while giving a working memory test. Ultimately, we found that working memory was not impaired during but after acute stress when cortisol levels were higher. We spent a lot of time designing the study, so both the cold pressor task and working memory test could be completed at the same time with resulting null results. Publishing these null results was important, however, so other researchers can save time and effort in the design of their studies.

Learning how to conduct mediation and moderated models has proved to be extremely useful. Thinking about how and why we see change and having the ability to test these relationships have pushed us to think about questions in different ways. In addition, being hands-on for all aspects of data collection and analysis, including learning how to measure cortisol levels, helped for future research as some of the potential pitfalls are now clearer.

Exercises and Discussion Questions

1. To what extent do you agree with the use of healthy college students to make inferences about clinical populations? What are the advantages and disadvantages?

2. Consider the method that you would use to assess anxiety, stress, and cognition. Explore the underlying theory behind the method and discuss how this would affect the data collection.
3. The first author of this project helped design the study, recruited and ran participants, and conducted data analyses. What are the benefits and what are the potential pitfalls for this strategy? What other approaches could have been used?
4. What statistical methods would you have chosen if you had conducted this study? Do you feel that moderated mediation was appropriate? Would you have used the bootstrapping method or is there another appropriate statistical technique?

Further Reading

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