Evaluating Respiratory Sinus Arrhythmia as a Mediator of Relations between Minority Stress and Depressive Symptoms Among Sexual Minorities

Research Thesis

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Abstract

Compared with heterosexuals, those with sexual minority identities (e.g., lesbian, gay, bisexual) are at significantly greater risk of psychopathology, including depression—the most common mental health disorder in the general population. A large body of research suggests that disparities in depression between heterosexuals and sexual minority groups are due, at least in part, to minority stress. Minority stress includes stressors unique to minority groups, such as marginalization and discrimination based on race or sexual identity. To date, most work demonstrating associations between minority stress and depression has used retrospective self-reports of minority stress exposure, so little is known about acute effects of minority stress exposure on physiological functions. In this study, I examine the potential moderating or mediating effect of respiratory sinus arrhythmia (RSA)—the high frequency component of heart rate variability associated with breathing—on associations between minority stress and symptoms of depression. Heterosexual (n = 43; 62.5% women) and sexual minority (n = 24; 59.3% women) college students viewed films designed to induce minority stress and completed the Beck Depression Inventory (BDI-II). Results indicated no differences in RSA reactivity or BDI scores between heterosexuals and sexual minority group members.

Those who identify as sexual minorities, including lesbian, gay, and bisexual individuals, are at greater risk for psychopathology compared to heterosexuals (Meyer, 2003). For example, sexual minorities experience higher rates of anxiety, depression, and suicidality (King et al., 2008; Plöderl & Tremblay, 2015). In a meta-analysis, risk for depression and anxiety were 1.5 times higher among sexual minorities (King et al., 2008). Furthermore, sexual minority college students report higher rates of lifetime depression (31.6%) than their heterosexual peers (16.7%) (Lindsey, Fabiano, & Stark, 2009), and, sexual minorities are 2-7 times more likely to attempt suicide (Haas et al., 2011; King et al., 2008). These findings are consistent even when controlling for age, race, and gender (Silenzio, 2007). For example, across the United States (Borowsky, Ireland, & Resnick, 2001) and Canada (Saewyc et al., 2007), sexual minority youth displayed elevated risk for suicidal ideation and attempts compared to heterosexuals. Overall, previous research highlights that sexual minorities experience greater mental health burden compared to heterosexual individuals, with disparities especially prevalent for depression.

Defining Sexual Minority Status

Sexual minorities are generally defined as individuals with non-heterosexual identities (e.g., lesbian, gay, bisexual, queer), sexual behaviors, or romantic attractions (Math & Seshadri, 2013). It is possible for individuals to report behaviors or attractions that are concordant or discordant from the sexual orientation that they report (Krueger, Meyer, & Upchurch, 2018). The diversity of representations of sexuality makes categorizing sexual minorities and non-sexual minorities a more complicated process for researchers. It also calls into question whether a participant who, for example, reports the label of "exclusively heterosexual," should be placed in the non-sexual minority group, even if he or she reported having non-heterosexual attractions or behaviors.

Since the present study focused on the self-reported sexual orientation variable, participants who reported any inconsistent sexual orientation dimension were placed in the heterosexual group.

Minority Stress Theory

A number of theories seek to explain the wide-ranging disparities seen among sexual minorities, including increased risk for depression. Minority stress theory (Meyer, 2003) suggests that individuals who identify as sexual minorities experience unique stressors related to sexual orientation that heterosexual peers do not. These stressors, collectively known as *minority stress*, may explain the increased risk for depression among sexual minorities (Luk et al., 2018). Meyer (2003) notes that minority stress processes include a mix of "stigma, prejudice, and discrimination [that] create a hostile and stressful social environment that causes mental health problems" (p. 1). Identifying features of minority stress are that it may be acute, chronic, and persist throughout the lifespan (Parent et al., 2019). Minority stress is often conceptualized as a combination of distal and proximal stressors that impact daily life (Meyer, 2003). Distal stressors are objective and independent of an individual's appraisals of the event (e.g., being called a slur), whereas proximal stressors are more subjective and dependent on identification as a sexual minority (e.g., concealment of sexual orientation for fears of rejection/discrimination) (Meyer, 2003).

Depressive Symptoms among Sexual Minorities

A number of studies have found associations between minority stress and depression among sexual minorities. On average, sexual minority individuals report more depressive symptoms than heterosexual individuals (Krueger, Meyer, & Upchurch, 2018;).

Sexual minorities report increased perceived burdensomeness, which is one mediating variable explaining increased depressive symptoms in sexual minorities (Baams, Russell, &

Grossman, 2015). Although limited sample sizes sometimes necessitate examination of sexual minorities as a homogenous group, there may be important differences in depression outcomes among sexual minorities. For example, some studies find that bisexual individuals report increased minority stress and depression compared to gay and lesbian individuals due to decreased family support, increased childhood adversity, and increased adverse life events (Jorm et al., 2002). However, others have found similar rates of minority stress between different sexual minority groups (Paveltchuk, Damásio, & Borsa, 2019).

Sex Differences in Mental Health Outcomes among Sexual Minorities

Previous work investigating sexual minorities and mental health outcomes has found that sex differences exist between men and women. In general, young women have double the prevalence of depression compared to young men (Patten et al., 2006). Several behavioral and biological factors may contribute to sex differences including daily habits, differing levels of hormones and neurotransmitters, and differing reward mechanisms (Ryba et al., 2012). One area that has been less explored is laboratory-based stress induction and differences in stress response between males and females. Past research has utilized stress induction tasks such as the Trier Social Stress Test. Following this task, males tend to have higher cortisol levels compared to women (Liu et al., 2017). However, results have failed to find a difference between the autonomic reactivity between men and women (Kelly et al., 2008).

Sexual Identity and Risk for Psychopathology

Sexual minorities are at higher risk of developing psychopathologies compared to heterosexual individuals. Specifically, sexual minority women are more likely to experience 12-month prevalence of major depression than heterosexual women (Gilman et al., 2001). In addition, sexual minority women are more likely to experience more stressful life events

compared to heterosexual women (Austin et al., 2016). Meanwhile, some findings suggest that sexual minority men are not statistically more likely to have major depression compared to heterosexual men, while other results show that gay men are at three times higher risk of depression compared to the general population and that homosexually active men are more likely to have major depression (Cochran & Mays, 2000). Men who report having a same-sex partner had a higher instance of anxiety, mood, or substance disorders (King et al., 2008). Taken together, these results suggest that sexual minorities have higher rates of psychiatric disorders, including suicidal behaviors (Fergusson et al., 1999). Emotion regulation has been cited as one mechanism that explains minority stress and the disproportionality of substance and alcohol use in this group (Rogers et al., 2017). Here, we use RSA as an indicator of emotion regulation and risk for psychopathology in sexual minorities due to its relation to the autonomic nervous system.

Physiological Research Among Sexual Minorities

A small body of work has examined health disparities among sexual minorities at the physiological level. In the general population, decades of work have elucidated effects of chronic stress on health. Stress describes any physical or mental state that alters the body's internal balance (Stephens & Wand, 2012). Chronic stress can lead individuals to carry a heavy allostatic load or cumulative damage caused by prolonged exposure to stress (Mays, Juster, Williamson, Seeman, & Cochran, 2018). In the long term, persistent stress can lead to elevations in essential objective measures such as blood pressure and heart rate (Parent et al., 2019). Elevated allostatic load was found in bisexual men when compared to that of their heterosexual peers. (Mays et al., 2018). No differences were found in the hypertensive risk between heterosexual/bisexual participants and heterosexual participants (Everett and Mollborn, 2013). However, there is less

work studying the effects of sexual minority stress and heart rate through autonomic mechanisms.

The hypothalamic-pituitary-adrenocortical (HPA) axis is one of the most well-documented examples of a homeostatic control mechanism (Austin et al., 2016). The HPA axis releases cortisol, a major hormone used to indicate varying levels of stress. Sexual minorities display a blunted cortisol response when they are "out," or have disclosed their sexual orientation at work (Huebner &Davis, 2005). Blunted cortisol responses have been documented in chronically stressed groups, including maltreated children (Ouellet-Morin et al., 2011). Similarly, sexual minority youth who grew up in stigmatized environments exhibited a decreased cortisol response when exposed to the Trier Social Stress Test, where participants anticipate giving an impromptu mock interview, and are asked to perform mental arithmetic (Hatzenbuehler & McLaughlin, 2014). Researchers hypothesize that the additional adversity sexual minorities go through may create an increased resilience to stress, which modulates HPA axis functioning in LGB young adults. However, more work studying autonomic functioning related to sexual orientation is needed to understand how minority stress interacts with mental health at a psychophysiological level. Nearly all studies of minority stress are self-reported and correlational, so little is known about acute responses to minority stress. Questions that remain include (1) how does acute exposure to a minority stress event affect the individual's bodily responses to stress, and (2) how does sexual minority stress play a role in the risk for depression symptoms compared to heterosexual individuals?

Differences in Emotion Regulation and RSA

Little work has been done in the area of emotion regulation and sexual minority mental health. However, it is known that sexual minority individuals experience additional stressors

related to their identities compared to heterosexual individuals. For example, sexual minority men experience a greater amount of emotion dysregulation due to internalized homonegativity and peer rejection (Pachakis et al., 2015). Sexual minorities tend to use different coping strategies compared to heterosexual individuals. For example, sexual minorities have been found to have increased ruminative tendencies compared to heterosexual individuals, especially when faced with stigma (Hatzenbuehler et al., 2009).

Supplementing self-report data with psychophysiological data may be helpful in developing a more complete picture of sexual minority health disparities, including vulnerability to depression. Heart rate variability, defined as changing lengths of times between heart beats across successive respiratory cycles (Kim et al., 2018), is an indicator of emotion regulation and psychological well-being (Paniccia et al., 2017). Previous studies found that reduced heart rate variability may be a predictor of death or act as a biomarker that can help identify vulnerability to various psychopathologies. Research shows that reduced HRV is associated with anxiety disorders (Chalmers et al., 2014), reduced ability to cope with stressors (Kim et al., 2018), and major depressive disorder (Gorman and Sloan, 2000). A potentially promising transdiagnostic biomarker of mental health is respiratory sinus arrhythmia (RSA). RSA is a specific component of HRV. RSA describes variation in heart rate due to respiration—heart rate increases during inhalation and heart rate decreases during exhalation (Beauchaine et al., 2019). Alterations in RSA are associated with both physical and mental health outcomes (Beauchaine & Thayer, 2015; Price & Crowell, 2016). RSA has been used as a measure of emotion regulation in individuals with substance use disorders (Price & Crowell, 2016). Low resting RSA is associated with several disorders and maladaptive behaviors, including depression, schizophrenia, and nonsuicidal self-injury (Beauchaine et al., 2019). Low resting RSA is associated also associated

with symptoms of borderline personality disorder and history of personal violence (Thomson & Beauchaine, 2018). Additionally, low resting RSA was associated with externalizing and internalizing disorders (Zhang, Fagan, & Gao, 2017). RSA has also been implicated in specific depression symptoms, including sadness and suicidality (Rottenberg, Wilhelm, Gross, & Gotlib, 2002). The resting RSA measure has been able to predict depression (Yaptangco et al., 2015).

While these diverse findings build a solid foundation outlining minority stress and depression outcomes, little is known about the connections between minority stress and psychophysiological variables such as RSA. Several studies have utilized emotion-inducing films to manipulate the affective, or emotional, states of participants in a controlled laboratory setting (Panaite et al., 2016). Past research highlights that emotion-inducing films can change autonomic activity including heart rate and skin conductance (Hubert & Jong-Meyer, 1991).

The Present Study

To date, there is a gap in the literature between what is known about depressive symptoms in sexual minorities and psychophysiological stress reactivity. This study examined minority stress theory under a new lens by inducing psychophysiological stress using contrasting video content. We hypothesized (1) that lesbian, gay, and bisexual students would have lower RSA in response to the stress-inducing video, and (2) that this increased minority stress would predict reports of more depressive symptoms on the Beck Depression Inventory II.

Method

This study was approved by the Institutional Review Board at The Ohio State University and participants provided informed consent before beginning the study.

Participants

Data collection occurred between February and December 2019. Participants were adults (N = 67, Mean age = 19.55 years, SD = 2.51) recruited from a pool of introductory psychology courses at a large Midwestern university. Eleven participants were excluded due to technical difficulties and/or incomplete data. Most participants identified as White (non-Hispanic ethnicity). Full demographics are listed in Table 1. This study used prescreening to overrecruit sexual minority participants (described below). Participants self-identified as heterosexual (n = 43), or lesbian, gay, bisexual (n = 24).

Prescreening

Participants reported self-identified sexual orientation in prescreening with the question, "What is your sexual orientation?" Responses included "heterosexual," "gay," "lesbian," or "bisexual." Sexual minority participants were informed of their eligibility for participation in the study and could voluntarily sign up for the study if interested. Participants received course credit and monetary compensation based on task performance earned from a reward task used as part of a larger study.

Measures

Participants responded to questionnaires presented via computer in a private, sound-attenuated room. Participants self-reported sexual orientation and the nature of their past sexual behavior as part of a questionnaire. Participants were asked to describe nature of their past sexual relationships as one of the following: "equally homosexual and heterosexual," "exclusively heterosexual," exclusively homosexual," "predominately heterosexual, but more than incidentally homosexual," "predominantly homosexual, but more than incidentally heterosexual," or "predominately homosexual, only incidentally heterosexual."

In this analysis, participants who reported heterosexual identities and LGB sexual behaviors (discordant sexuality domains) were grouped as heterosexuals, while participants that reported congruent heterosexual identities and behaviors were grouped as sexual minority participants. Three participants fell into the discordant categories as the sexual identity they reported was different than the nature of their past sexual relationships. For example, one participant reported heterosexual identity but "predominately heterosexual, only incidentally homosexual" past relationships, and another participant reported heterosexual identity but "equally homosexual and heterosexual." Two participants reported having "another sexual orientation" and described being asexual with "no past sexual relationships" and queer with "predominately heterosexual, only incidentally homosexual" past relationships. Including these participants, the sexual minority group compromised 24 participants and the heterosexual group included 43 participants.

Beck Depression Inventory

The Beck Depression Inventory (BDI-II) was used to measure depressive symptoms such as sadness and suicidal thoughts. The measure consists of 21-items reported on a Likert-type scale from 0 to 3 (total scores range from 0 to 63). Higher scores indicate more depressive symptoms (Beck, Steer, R.A., & Brown, 1996). The BDI has high internal consistency among college students (Cronbach's alpha= 0.93) and test-retest reliability ranging from 0.60-0.93 (Smarr et al., 2011).

Respiratory Sinus Arrhythmia (RSA)

This study design was modeled from previous research differentiating psychophysiological data into two domains: psychophysiological activity (at rest) and psychophysiological reactivity (change in rest) showing that individuals with depression exhibited lower RSA (Beauchaine,

Gartner, & Hagen, 2000; Beauchaine, 2015; Rottenberg, 2002; Pang & Beauchaine, 2013). Psychophysiological data were recorded using noninvasive BIOPAC® module hardware throughout the duration of the 2-hour experiment. Specifically, RSA was measured to assess tonic responses to two videos: a stress video and a neutral video. In order to collect valid data from psychophysiological software, baselines were measured at the beginning of each trial of the experiment to reduce sources of error associated with psychophysiological data (Beauchaine & Hinshaw, 2008). Participants were counterbalanced to the type of video stimuli they watched first during the study, either neutral or stress-inducing. The neutral stimuli presented a dynamic city landscape video. The stress stimuli presented a compilation video in which parents, teachers, and political figures disparaged LGB culture and lifestyle. The stress stimulus was validated in a previous study to induce a minority stress response (Seager, 2016).The RSA analysis was performed using the average RSA reactivity recorded during the stress video compared to the average RSA reactivity during the stress baseline recording.

Data Analyses

Data were analyzed using SPSS 25 (IBM Corporation, Armonk, NY, United States) and the PROCESS SPSS macro version 3 (Hayes, 2018). A change score was computed by taking the difference between the post-induction stress and pre-induction stress baseline as part of a larger study (Hahn, Seager, & Ahn, 2020). In the mediation model, depression score served as the dependent variable, group (sexual minority vs. heterosexual) acted as the independent variable, and RSA acted as the mediating variable. Kubios HRV software (Kuopio, Finland) was used to transform inter beat interval (IBI) files into HRV analysis compatible data to assess autonomic nervous system function.

Procedure

Participants were run individually by trained research assistants. Each participant gave informed consent in a private room with a television monitor, camera, desk, and chair. An ambient noise machine was turned on during each session to minimize noise heard from other offices. Electrodes and leads (BIOPAC® Systems, Inc) were attached to the neck, chest, abdomen, left hand, middle, and index fingers of each participant. Additionally, the participant wore a respiration belt and ECG modules around the waist. Using a standardized script, an experimenter instructed the participant on how to communicate using a two-way speaker in the lab. Participants sat in front of a computer and were asked to be as still as possible and relax while a baseline was recorded. The experimenter sat in a different room with a dual monitor to visualize the pattern of the physiological output as well as the movement of the participant simultaneously. Participants completed a reward task followed by several surveys asking about demographics, sexual orientation, and depression (BDI-II) online using Qualtrics software. Physiological data were recorded throughout the duration of the study using the AcqKnowledge Data Acquisition & Analysis Program (BIOPAC® Systems, Inc). After completing a battery of assessments, participants were randomized to view two separate video stimuli including a previously validated two-minute video containing homophobic content (Seager, 2016) to induce a minority stress response, and a two-minute video depicting a walking tour of London, United Kingdom (Hahn et al., 2020). Once the study concluded, participants were provided with debriefing and mental health resources.

Results

Demographic information is presented in Table 1.

Table 1. *Demographics*

		Sexual Minority		Hetero sexual		Total	
		n	%	*n*	%	*n*	%
		2	38.5	43	61.4	67	85.9
Race/ Ethnicity							
	White	24	88.9	27	67.5	51	65.4
	African American	1	3.7	0	0	1	1.3
	Asian	1	3.7	10	25	11	14.1
	Mixed Race/ Other	1	3.7	2	5	3	3.8
Sexual orientation							
	Hetero sexual	0	0	40	100	40	59.7
	Gay or lesbian	6	9	0	0	6	9
	Bisexual	18	26.9	0	0	18	26.9
		M	*S. D.*	*M*	*S. D.*	*M*	*S. D.*
Age		19.3	3.30	19.60	1.87	19.55	2.51

Four statistical models were constructed based on PROCESS (Hayes, 2018). The first model depicted mediation of baseline RSA between sexual orientation and depression (See Fig 1).The second model included moderation of baseline RSA between sexual orientation and depression (See Fig 2). The third model depicted mediation of RSA reactivity to the stress video between sexual orientation and depression (See Fig 3). The fourth model included RSA

reactivity in response to the stress video as the moderator between sexual orientation and depression (See Fig. 4).

Table 2 shows means and standard deviations for baseline RSA, RSA reactivity, and total scores on the *BDI-II* for participants separated by group. Results indicate that baseline RSA values did not differ between the heterosexual (M = 6.54, *SD* = 1.19) and sexual minority group (M = 6.36, *SD* = 1.57), $t(63) = .498$, $p = .255$. In addition, change scores used to derive RSA reactivity did not suggest a significant difference between the heterosexual (M = .17, *SD* = .62) and the sexual minority group (M = .04, *SD* = .74), $t(64) = .79$, $p = .25$. Finally, mean depression scores were slightly lower in the different the heterosexual group (M = 11.75, *SD* = 8.26) compared to the sexual minority group (M = 13.35, *SD* = 9.75), however, this relationship did not reach significance $t(60) = -.696$, $p = .330$

Table 2. *Descriptive statistics: RSA values and BDI scores.*

	Baseline RSA		RSA Reactivity		*BDI* Total	
	Mean	*SD*	Mean	*SD*	Mean	*SD*
Heterosexual	6.54	1.19	.17	.62	11.75	8.26
Sexual minority	6.36	1.57	.04	.74	13.35	9.75

Baseline RSA

Six participants had missing BDI data and were excluded from the analysis for a final sample of $N = 61$. The overall model was not significant in predicting depression scores from sexual orientation, baseline, and their interaction, $F(3, 57) = .40$, $p = .76$, $R^2 = .02$.

The relationship between sexual orientation and baseline RSA was not significant $F(1, 57) = .36$, $p = .55$. The relationship between sexual orientation and baseline RSA was not significant $b = 2.07$, $p = .37$, 95%CI: [-2.54, 6.69].The relationship between baseline RSA and depression was not significant $b = .233$, $p = 0.79$, 95% CI: [-1.56, 2.02]. The direct effect of sexual orientation on depression scores was not significant, $b = 2.07$, $p = .37$, 95% CI: [-2.55, 6.70] and the indirect effect of baseline RSA was not significant $b = -0.69$, 95% CI: [-.94, .95].

RSA Reactivity

Five participants had missing BDI data and were excluded from the analysis for a final sample of $N = 62$. The overall model was not significant in predicting depression scores from sexual orientation, RSA reactivity, and their interaction, $F(3, 58) = 1.01$, $p = .39$, $R^2 = .05$. There was no significant interaction between sexual orientation and RSA reactivity, $F(1,58) = 0.008$, $p = .93$. The relationship between sexual orientation and RSA reactivity was not significant $b = 0.11$, $p = .52$, 95% CI: [-0.46, 0.24]. Similarly, the relationship between RSA reactivity and depression was not significant $b = 2.69$, $p = .11$, 95% CI: [-0.66, 6.04]. The direct effect of sexual orientation on depression scores $b = 1.90$, $p = .41$, 95% CI: [-2.65, 6.45] and the indirect effect of RSA reactivity was not significant $b = -0.30$, 95% CI: [-1.69, 0.83].

Discussion

Depression is a mental health disorder that affects 16.1 million people in the United States (Anxiety and Depression Association of America, n.d.). It is an important mental health condition to study because it is the leading cause of disability and also is the leading cause of death by suicide (Bachmann, 2018). College students are especially prone to develop depression because of increased demands that may be due to lack of social support, finances, (Adams, Meyers, & Beidas, 2016) and increased academic rigor (Pedrelli et al., 2015). According to

minority stress theory, sexual minority individuals are at higher risk of developing depression for a variety of reasons both related to their sexual minority status and unrelated to it (Meyer, 2003). My study used the minority stress theory as a model to examine whether sexual orientation predicts baseline RSA and/or RSA reactivity, as well as whether RSA mediated or moderated associations between sexual orientation and depression in college students. The aim of the study was to elucidate a potential parasympathetic nervous system mechanism that influences depression scores in sexual minorities and heterosexual individuals. Neither of the hypotheses were supported as findings revealed no significant relationship between sexual orientation, baseline RSA/RSA reactivity, and depression scores.

However, we expected to find reduced baseline RSA among sexual minority individuals because lower resting RSA is documented among those with mental health problems (Gorman and Sloan, 2000; Chalmers et al., 2014; Hamilton & Allot, 2017), such as depression, anxiety, substance abuse, and suicide attempts, which are observed at higher rates among sexual minorities (King et al., 2008).

The stress video used in our study, which was composed of discriminatory remarks, was chosen as the stimulus because it was previously validated as a stimulus that induces a minority stress response. In addition, the video has high external validity since it utilized 40 clips from multiple sources, including YouTube and news channels (Seager, 2016). Since this video was designed as a minority stress response stimulus, we expected to see lower RSA reactivity in response to this derogatory video in the sexual minority group compared to changes in RSA reactivity for the heterosexual group. In turn, we expected higher depression scores for the sexual minority group. This prediction is based on past research that found reduced RSA reactivity in depressed individuals (Rottenberg et al., 2002). This idea was also informed by the minority

stress model, which suggests that minority populations endure additional stressors related to their sexual orientation (e.g., coming out stress, identity disclosure, etc.) that may contribute to negative mental health problems (Meyer, 2003).

Taken together, these findings would suggest that sexual minorities are more likely to display reduced RSA reactivity and higher depression scores. However, different studies examining RSA and its relation to mental health outcomes have found an opposite or no effect. For example, one study found that a higher RSA at time one predicted non-recovery from depressive symptoms at time two (Rottenberg et al., 2002). Our null findings are inconsistent with that of past RSA research showing reduced RSA levels in depressed individuals and may be due to several factors.

In the mediation and moderation analyses, results indicated that sexual orientation was not associated with either (a) baseline RSA or RSA reactivity, or (b) depression. First, the interaction between sexual orientation and baseline RSA was not significant. Second, RSA reactivity was not a variable that affected the strength of the relationship between minority stress (the sexual orientation variable) and depression scores.

These results indicate that neither mediation nor moderation occurred in the sample, and that RSA may not be the mechanism which determines the direction or strength of the relationship between sexual orientation and depression scores in sexual orientation. Because the interactions between sexual orientation and RSA reactivity and baseline RSA were not significant, these results do not support my hypotheses nor the minority stress model overall. The results, which were null for all models, do not provide support to the minority stress theory. Null findings may be attributable to any of several factors which must be taken into consideration when interpreting the results.

The first limitation is the limited sample size of the study. The small sample size has the negative consequence of making the study underpowered, which decreases the confidence of conclusions that can be made based on the findings. Additionally, there were only 24 participants in the sexual minority group, and nearly double the number of participants in the heterosexual group. Another complicating factor related to the sample size is that RSA accounted for less than 5% of the variance in depression scores seen in the sample. With a sample of only 67 participants, it is difficult to have conclusive results on whether RSA mediated or moderated the relationship between sexual orientation and depression scores. As a result, in the future, researchers should conduct a power analysis at the beginning of the study design phase in order to determine the appropriate number of participants to run and plan more effective sexual minority recruitment strategies.

In addition, the generalizability of the results is limited because we did not use a random sampling procedure. Instead, we used a convenience sample to recruit participants who were enrolled at a large Midwestern university and completed the study for course credit. This may negatively influence the results of the study because it has been documented that studies utilizing student samples in psychological research tend to be more homogenous and incorrectly estimate personal variables such as sexual behaviors compared to the general public (Hanel & Vione, 2016).

Additionally, the practice of using student samples in the investigation of sexual orientation and depression may be problematic because in this study, students could volunteer to sign up for the experiment if they were eligible and completed the prescreening questionnaire. This has implications on the generalizability of the results because the students who did participate in our study may be more “out” and comfortable with their sexual orientation. This

may have an impact on the effectiveness of the stress manipulation using the derogatory and neutral videos. One potential problem may be that participants who volunteered for the study displayed a different RSA reactivity compared to what would be expected in sexual minority individuals within the general population if they were chosen to participate randomly. The last participant-related factor that may contribute to the null findings is due to the sample population. Since only college students were tested, we are unable to generalize these conclusions about depression outcomes in sexual minority individuals who are not currently enrolled in university.

The minority stress model applies to a variety of minority identities, including sexual orientation, socioeconomic status, gender, and race/ethnicity (Meyer, 2003). While this study focused on the sexual orientation of participants in the context of the minority stress theory, it is possible that the demographics of our study (mostly white, educated young adults enrolled in a university) further explain the null findings that were seen. Overall, the lack of racial and ethnic diversity among the tested sample may not be representative of the entire sexual minority community. Additionally, the lack of diversity is a factor that may affect interpretation of results because holding multiple identities (e.g., Asian Bisexual male) may play a role in the appraisal of negative stimuli, due to the increased experience of proximal stressors on a daily basis (Meyer, 2003). More recent research investigating diverse populations found that Black sexual minority men reported higher racial/ethnic stigma in LGBT spaces compared to White sexual minority men (McConnell et al., 2018). Additionally, sexual minority women of color were more likely to report not being “out” compared to White sexual minority women (Balsam et al., 2015). African American and Latino sexual minority participants experience increased stigma compared to White sexual minority participants (Shangani et al., 2020). All of these findings support the idea that the minority stress model may be dynamic and affected by holding multiple minority

identities. An area that is less researched is the connections between RSA reactivity, racial and ethnic diversity, and in turn, depression scores in sexual minorities. By investigating these areas, the minority stress theory may be further evaluated using larger and more diverse sample sizes to better match the U.S. population demographics of sexual minority individuals. Both of these changes would help strengthen the theory by adding empirical evidence from a more representative sample.

Additionally, another possible explanation of the results is that the derogatory video used to invoke a sexual minority stress response may not have been appropriate to induce changes in the RSA reactivity measure specifically. The video was chosen as the stress stimulus and was previously validated to induce a minority stress response (Seager, 2016). However, the video was originally tested in Amazon Mechanical Turk workers who were not asked about depressive symptoms or attached to psychophysiological sensors to assess RSA reactivity in response to the stress video. This would explain why there was not a significant difference in RSA reactivity seen between the heterosexual and sexual minority groups.

Membership in a sexual minority group may have numerous consequences, both positive and negative. Sexual orientation was the primary independent variable that was focused on in this study. However, it is only one component of sexual identity. Future studies could look at how different components of sexual identity have an impact on depressive symptoms, as it is possible for someone to identify with a certain sexual orientation and engage in sexual behaviors or have sexual attractions that are different from their orientation.

The goal of our study was to examine the effects of an acute stressor on RSA in a laboratory setting. In the future, something researchers can look at is whether sexual minority participants experience increased depressive symptoms over a longer period of time, such as over

the period of one week, 6 months, and one year as in (Switzer et al., 2018). In the future, research related to minority stress theory would benefit from using longitudinal methods to assess the long-term impacts of stress related to sexual orientation and mental health struggles. Using a longitudinal study design, including structured interviews and assessments of depressive symptoms at multiple time points, would allow researchers to investigate what factors, if any, contribute to the elevated risk of depression and suicide attempts in sexual minority individuals.

Another interesting area of research to explore in the future is resilience and factors that buffer against depressive symptoms specifically in the sexual minority population. A surprising finding is that sexual minority students are actually more likely to experience increased depression when they are involved with LGBQ clubs and activities compared to students who are not involved with similar organizations (Bissonette, 2018). This finding was attributed to the possibility that by having membership in LGBQ campus activities, students may be "outing" themselves and thus increasing the risk that they experience discrimination.

The strengths of this study include the experimental study design as participants were randomly assigned to watch the neutral video or stress video. Another strength of the design is utilizing multiple methods to assess the variables of interest. By combining survey methods with RSA, a psychophysiological measure, we were able to contribute an additional level of analysis, which is in line with the principles of the Research Domain Criteria. Findings are not consistent with past studies using different methods. This includes studies that strictly use survey questionnaires to assess depression among sexual minority individuals and studies that make use of an experimental design such as the Trier Stress Test, which is also implicated in activating a parasympathetic response.

Major limitations of our study include the small sample size, lack of ethnic/racial diversity among participants, and the convenience sampling method used to recruit participants. Considering all of these pitfalls, it is premature to make the final determination that there is no relation between RSA and depression scores in sexual minorities at this time. Still, the null results of the study contribute to the literature by initiating a novel discussion about sexual orientation, RSA, and depressive symptoms. This study contributed to the literature by a) implementing an experimental design in the evaluation of RSA, and b) studying depressive symptoms among an underrepresented group, sexual minorities. Because RSA is known to be a predictor of mental health outcomes in other populations, this measure should continue to be rigorously tested as a factor that may play a role in minority stress theory as a whole. While the results of the experiment were nonsignificant, researchers should work towards uncovering additional mediators and moderators that may shed light on disparities in minority mental health.

References

Adams, D. R., Meyers, S. A., & Beidas, R. S. (2016). The relationship between financial strain, perceived stress, psychological symptoms, and academic and social integration in undergraduate students. *Journal of American College Health, 64*(5), 362–370. doi:10.1080/07448481.2016.1154559

Anxiety and Depression Association of America. (n.d.). *Understand the Facts: Depression*. https://adaa.org/understanding-anxiety/depression

Austin, S. B., Rosario, M., McLaughlin, K. A., Roberts, A. L., Gordon, A. R., Sarda, V., … Scherer, E. A. (2016). Sexual orientation and diurnal cortisol patterns in a cohort of U.S. young adults. *Psychoneuroendocrinology*, *69*, 197-208. doi:10.1016/j.psyneuen.2016.04.012

Baams, L., Grossman, A. H., & Russell, S. T. (2015). Minority stress and mechanisms of risk for depression and suicidal ideation among lesbian, gay, and bisexual youth. *Developmental Psychology*, *51*(5), 688–696. doi:10.1037/a0038994

Bachmann, S. (2018). Epidemiology of Suicide and the Psychiatric Perspective. *International Journal of Environmental Research and Public Health*, *15*(7), 1425. doi:10.3390/ijerph15071425

Beauchaine, T. P., & Hinshaw, & S. P. (2008). *Child and Adolescent Psychopathology*. (3rd Ed.). Hoboken, NJ: Wiley.

Beauchaine, T. P. (2015). Respiratory sinus arrhythmia: A transdiagnostic biomarker of emotion dysregulation and psychopathology. *Current Opinion in Psychology, 3,* 43-47.

doi:10.1016/j.copsyc.2015.01.017

Beauchaine, T. P., & Thayer, J. F. (2015). Heart rate variability as a transdiagnostic biomarker of psychopathology. *International Journal of Psychophysiology, 98*, 338-350. 350. doi:10.1016/j.ijpsycho.2015.08.004.

Beck, A.T., Steer, R.A., & Brown, G.K. (1996). Manual for the Beck Depression Inventory-II. San Antonio, TX: Psychological Corporation.

Bissonette, D. L. (2018). Minority stress and LGBQ college students' depression: Roles of peer group and involvement. Master's thesis. The University of Tennessee, Knoxville, TN.

Borowsky, I. W., Ireland M., & Resnick M.D. (2001) Adolescent suicide attempts: Risks and protectors. *Pediatrics*, *107*, 485-4–49. doi: 10.1542/peds.107.3.485

Chalmers, J. A., Quintana, D. S., Abbott, M. J., & Kemp, A. H. (2014). Anxiety disorders are associated with reduced heart rate variability: A meta-analysis. *Frontiers in Psychiatry, 5*, 80. doi:10.3389/fpsyt.2014.00080

Chambers, A. S., Allen, J.J. (2002). Vagal tone as an indicator of treatment response in major depression. *Psychophysiology*, *39*(6), 861–864.

Cochran, S. D., & Mays, V. M. (2000). Relation between psychiatric syndromes and behaviorally defined sexual orientation in a sample of the US population. *American Journal of Epidemiology*, *151*(5), 516-–523. https://doi:.org/10.1093/oxfordjournals.aje.a010238

Everett, B., & Mollborn, S. (2013). Differences in hypertension by sexual orientation among U.S. young adults. *Journal of Community Health, 38*(3), 588–596. doi:10.1007/s10900-013-9655-3

Fergusson D. M., Horwood L. J., & Beautrais A. L. (1999). Is sexual orientation related to mental health problems and suicidality in young people? *Archives of General Psychiatry*,*56*, (10):876-–880. doi:10.1001/archpsyc.56.10.876

Gorman, J. M., Sloan, R. P. (2000). Heart rate variability in depressive and anxiety disorders. *American Heart Journal, 140,* 77-83. doi:10.1067/mhj.2000.109981.

Haas, A. P., Eliason, M., Mays, V. M., Mathy, R. M., Cochran, S. D., D'Augelli, A. R., … Clayton, P. J. (2011). Suicide and suicide risk in lesbian, gay, bisexual, and transgender populations: review and recommendations. *Journal of Homosexuality*, *58*, 10-51. doi:10.1080/00918369.2011.534038

Hamilton, J. L., & Alloy, L. B. (2017). Physiological markers of interpersonal stress generation in depression. Clinical Psychological Science, *5*(6), 911–929. doi:10.1177/2167702617720211

Hanel, P. H., & Vione, K. C. (2016). Do student samples provide an accurate estimate of the general public?. *PloS one*, *11*(12), doi:10.1371/journal.pone.0168354

Hatzenbuehler, M. L., McLaughlin, K. A., & Slopen, N. (2013). Sexual orientation disparities in cardiovascular biomarkers among young adults. *American Journal of Preventive Medicine, 44*(6), 612–621. doi:10.1016/j.amepre.2013.01.02

Hatzenbuehler, M. L., & McLaughlin, K. A. (2014). Structural stigma and hypothalamic- pituitary-adrenocortical axis reactivity in lesbian, gay, and bisexual young adults. *Annals of Behavioral Medicine, 47*, 39-47. doi:10.1007/s12160-013-9556-9

Hubert, W., Jong-Meyer, R. (1991). Autonomic, neuroendocrine, and subjective responses to emotion-inducing film stimuli, *International Journal of Psychophysiology, 11*(2), 131-140. doi:10.1016/0167-8760(91)90005-I.

Huebner D. M., Davis M. C. (2005). Gay and bisexual men who disclose their sexual orientations in the workplace have higher workday levels of salivary cortisol and negative affect. *Annals of Behavioral Medicine*, *30*(3), 260 267 doi:10.1207/s15324796abm3003_10

Jorm, A., Korten, A., Rodgers, B., Jacomb, P., & Christensen, H. (2002). Sexual orientation and mental health: Results from a community survey of young and middle – aged adults. *British Journal of Psychiatry*, *180*(5), 423-427. doi:10.1192/bjp.180.5.423

Kelly, M. M., Tyrka, A. R., Anderson, G. M., Price, L. H., & Carpenter, L. L. (2008). Sex differences in emotional and physiological responses to the Trier Social Stress Test. *Journal Of Behavior Therapy and Experimental Psychiatry*, *39*(1), 87–98. https://doi.org/10.1016/j.jbtep.2007.02.003

Kim, H. G., Cheon, E. J., Bai, D. S., Lee, Y. H., & Koo, B. H. (2018). Stress and heart rate variability: A meta-analysis and review of the literature. *Psychiatry Investigation, 15*, 235-245. doi:10.30773/pi.2017.08.17

King, M., Semlyen, J., Tai, S. S., Killaspy, H., Osborn, D., Popelyuk, D., & Nazareth, I. (2008). A systematic review of mental disorder, suicide, and deliberate self-harm in lesbian, gay and bisexual people. *BMC Psychiatry, 8*. doi:10.1186/1471-244X-8-70

Krueger, E. A., Meyer, I. H., & Upchurch, D. M. (2018). Sexual orientation group differences in perceived stress and depressive symptoms among young adults in the United States. *LGBT Health*, *5*(4), 242–249. doi:10.1089/lgbt.2017.0228

Lindsey, B. J., Fabiano, P., & Stark, C. (2009). The prevalence and correlates of depression among college students. *College Student Journal*, *43*, 999-1014.

Liu, J. J.W, Ein, N., Peck, K. & Huang, V. & Pruessner, J. C., & Vickers, K. (2017). Sex differences in salivary cortisol reactivity to the Trier Social Stress Test (TSST): A meta-

analysis. *Psychoneuroendocrinology. 82*. doi:10.1016/j.psyneuen.2017.04.007

Luk W. J., Gilman E. S., Haynie L. D., & Simons-Morton, B. G. (2018). Sexual orientation and depressive symptoms in adolescents. *Pediatrics*, *141*, e20173309. doi:10.1542/peds.2017-3309

Math, S. B., & Seshadri, S. P. (2013). The invisible ones: sexual minorities. *The Indian Journal of Medical Research*, *137*(1), 4–6.

Mays, V. M., Juster, R. P., Williamson, T. J., Seeman, T. E., & Cochran, S. D. (2018). Chronic physiologic effects of stress among lesbian, gay, and bisexual adults: results from the national health and nutrition examination survey. *Psychosomatic Medicine*, *80*(6), 551–563. doi:10.1097/PSY.0000000000000600

McConnell, E. A., Janulis, P., Phillips, G., 2nd, Truong, R., & Birkett, M. (2018). Multiple minority stress and LGBT community resilience among sexual minority men. *Psychology of Sexual Orientation and Gender Diversity*, *5*(1), 1–12. doi:/10.1037/sgd0000265

Meyer I. H. (2003). Prejudice, social stress, and mental health in lesbian, gay, and bisexual populations: Conceptual issues and research evidence. *Psychological Bulletin*, *129*, 674-697. doi:10.1037/0033-2909.129.5.674

Ouellet-Morin, I., Odgers, C. L., Danese, A., Bowes, L., Shakoor, S., Papadopoulos, A. S., Caspi, A., Moffitt, T. E., & Arseneault, L. (2011). Blunted cortisol responses to stress signal social and behavioral problems among maltreated/bullied 12-year-old children. *Biological Psychiatry, 70*(11), 1016–1023. doi:10.1016/j.biopsych.2011.06.017

Pachankis, J. E., Hatzenbuehler, M. L., Rendina, H. J., Safren, S. A., & Parsons, J. T. (2015). LGB-affirmative cognitive-behavioral therapy for young adult gay and bisexual men: A

randomized controlled trial of a transdiagnostic minority stress approach. *Journal of Consulting and Clinical Psychology*, *83*, 875-889. doi:10.1037/ccp0000037

Panaite, V., Hindash, A. C., Bylsma, L. M., Small, B. J., Salomon, K., & Rottenberg, J. (2016). Respiratory sinus arrhythmia reactivity to a sad film predicts depression symptom improvement and symptomatic trajectory. *International Journal of Psychophysiology*, *99*, 108–113. doi:0.1016/j.ijpsycho.2015.12.002

Pang, K. C., & Beauchaine, T. P. (2013). Longitudinal patterns of autonomic nervous system responding to emotion evocation among children with conduct problems and/or depression. *Developmental Psychobiology, 55,* 698-706. doi:10.1002/dev.21065

Paniccia, M., Paniccia, D., Thomas, S., Taha, T., & Reed N. (2017). Clinical and non-clinical depression and anxiety in young people: a scoping review on heart rate variability. *Autonomic Neuroscience: Basic and Clinical.* 208:1–14. doi: 10.1016/j.autneu.2017.08.008

Parent, M. C., Arriaga, A. S., Gobble, T.,& Wille, L. (2019). Stress and substance use among sexual and gender minority individuals across the lifespan. *Neurobiology of Stress*, *10*, 2352-2895. doi: 10.1016/j.ynstr.2018.100146

Patten, S. B., Wang, J. L., Williams, J. V., Currie, S., Beck, C. A., Maxwell, C. J., & el-Guebaly, N. (2006). Descriptive epidemiology of major depression in canada. *The Canadian Journal of Psychiatry*, *51*(2), 84–90. doi:10.1177/070674370605100204

Paveltchuk, F. O., , Damásio, B. F., & Borsa, J. C. (2019). Impact of sexual orientation, social support and family support on minority stress in LGB people. *Trends in Psychology*, *27*(3),

735-748. doi: 10.9788/TP2019.3-10

Pedrelli, P., Nyer, M., Yeung, A., Zulauf, C., & Wilens, T. (2015). College students: mental health problems and treatment considerations. *Academic Psychiatry*, *39*(5), 503–511. doi:10.1007/s40596-014-0205-9

Plöderl, M. & Tremblay, P. (2015). Mental health of sexual minorities. A systematic review*, International Review of Psychiatry*. *27*. 1-19. doi:10.3109/09540261.2015.1083949.

Price, C. J., & Crowell, S. E. (2016). Respiratory sinus arrhythmia as a potential measure in measure in substance use treatment--outcome studies. *Addiction (Abingdon, England)*, *111*(4), 615–625. doi:10.1111/add.13232

Rogers, A. H., Seager, I., Haines, N., Hahn, H., Aldao, A., & Ahn, W. Y. (2017). The indirect effect of emotion regulation on minority stress and problematic substance use in lesbian, gay, and bisexual individuals. *Frontiers in Psychology*, *8*, 1881. doi:10.3389/fpsyg.2017.01881

Rottenberg, J., Wilhelm, F. H., Gross, J. J., & Gotlib, I. H. (2002). Respiratory sinus arrhythmia as a predictor of outcome in major depressive disorder. *Journal of Affective Disorders*, *71*(1-3), 265–272. doi: 10.1016/S0165-0327(01)00406-2

Saewyc, E., Poon C., Wang N., Homma Y., Smith, A. (2007). The McCreary Centre Society. Not Yet Equal: The health of lesbian, gay, & bisexual youth in BC. Vancouver, BC: McCreary Centre Society.

Seager, I. (2016). Regulating discrimination: the effects of emotion regulation on experiences of pride and shame, and subsequent self-disclosure among lesbian, gay, and bisexual adults.

Master's thesis, The Ohio State University, Columbus, OH.

Shangani, S., Gamarel, K. E., Ogunbajo, A., Cai, J., & Operario, D. (2020) Intersectional minority stress disparities among sexual minority adults in the USA: the role of race/ethnicity and socioeconomic status, *Culture, Health & Sexuality*, *22*(4), 398-412, doi: 10.1080/13691058.2019.1604994

Silenzio, V. M., Pena, J. B., Duberstein, P. R., Cerel, J., & Knox, K. L. (2007). Sexual orientation and risk factors for suicidal ideation and suicide attempts among adolescents and young adults. *American Journal of Public Health*, *97*(11), 2017–2019. doi:10.2105/AJPH.2006.095943

Smarr, K. L., & Keefer, A. L. (2011). Measures of depression and depressive symptoms: Beck Depression Inventory-II (BDI-II), Center for Epidemiologic Studies Depression Scale (CES-D), Geriatric Depression Scale (GDS), Hospital Anxiety and Depression Scale (HADS), and Patient Health Questionnaire-9 (PHQ-9). *Arthritis Care and Research*, *63*, 454-466. doi:10.1002/acr.20556

Stephens, M. A., & Wand, G. (2012). Stress and the HPA axis: role of glucocorticoids in alcohol dependence. *Alcohol Research: Current Reviews*, *34*(4), 468–483.

Switzer, A., Caldwell, W., da Estrela, C., Barker, E. T., & Gouin, J. P. (2018). Dyadic coping, respiratory sinus arrhythmia, and depressive symptoms among parents of preschool children. *Frontiers in Psychology*, *9*, 1959. doi:10.3389/fpsyg.2018.01959

Thomson, N. D., & Beauchaine, T. P. (2018). Respiratory sinus arrhythmia mediates links

between borderline personality disorder symptoms and both aggressive and violent behavior. *Journal of Personality Disorders*, *32*, 1-16. doi:10.1521/pedi_2018_358

Figure 1. PROCESS Model 4 with Baseline RSA as a mediator of the relation between sexual orientation and depression scores.

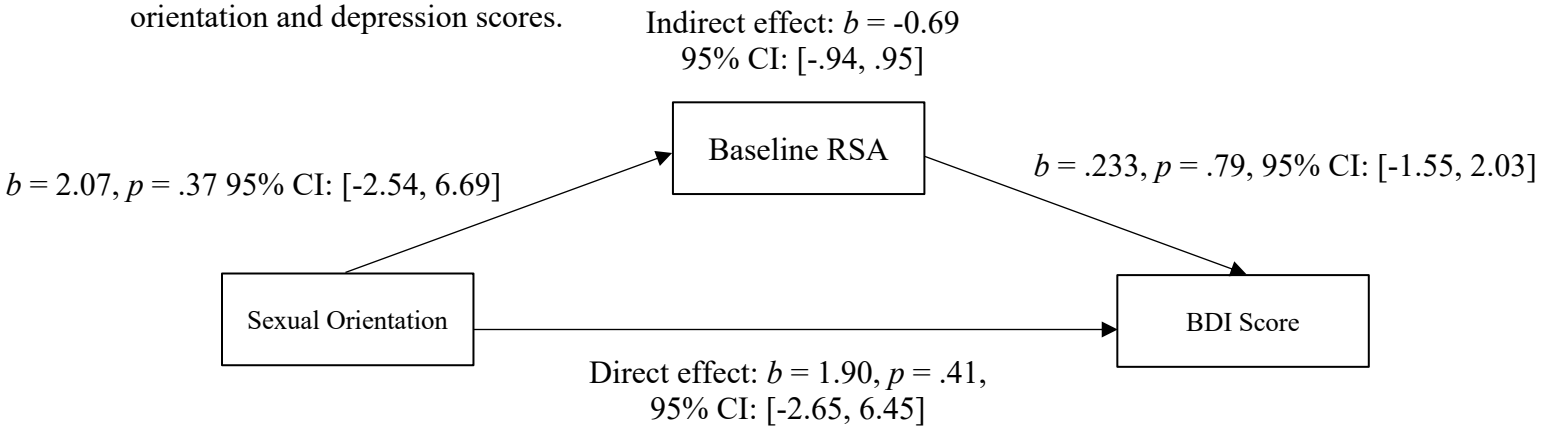
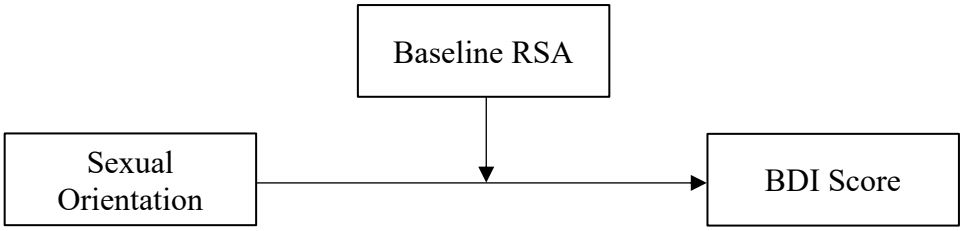


Figure 2. PROCESS Model 1 with Baseline RSA as a moderator of the relation between sexual orientation and depression scores



$F(1, 57) = 0.008$, $p = .55$

Figure 3. PROCESS Model 4 with RSA Reactivity as a mediator of the relation between sexual orientation and depression scores.

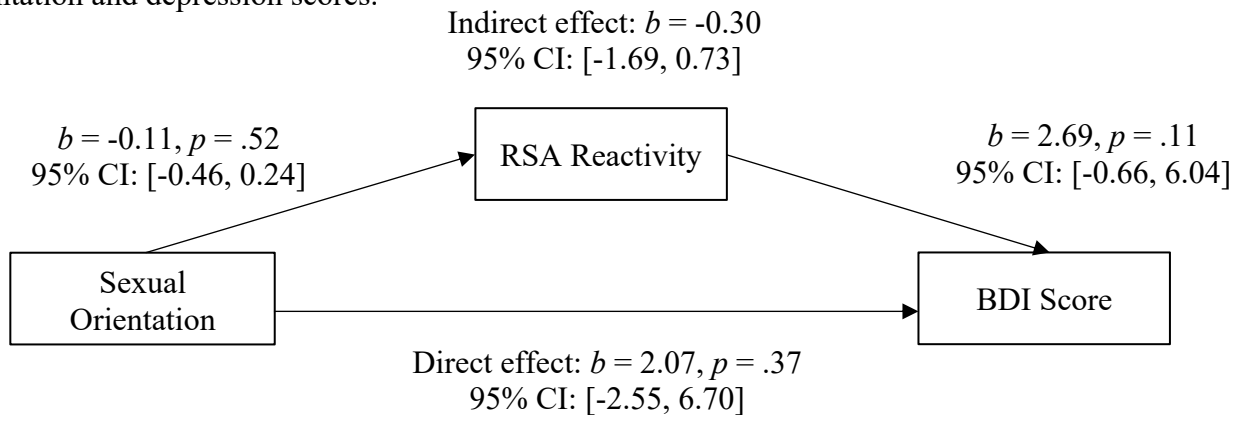


Figure 4. PROCESS Model 1 with RSA Reactivity as a moderator of the relation between sexual orientation and depression scores.