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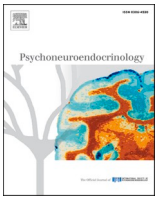

Dysregulated by stigma: Cortisol responses to repeated psychosocial stress in gay and heterosexual men

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ABSTRACT

Background: Research on pathways linking stigma with health inequalities affecting sexual minority populations, focused predominantly on exploring the hypothalamic-pituitary-adrenocortical (HPA) dysregulation profiles associated with chronic stress. One of such profiles reflecting a state of increased susceptibility to disease, and not yet studied among sexual minority individuals, is impaired habituation to repeated stress of the same type. In this study we explored whether sexual identity modulates endocrine stress responses and stress responses habituation in healthy heterosexual and gay men. We also explored the associations between perceived sexual minority stigma and cortisol response to stress in the latter group.

Methods: Gay (N = 49) and heterosexual (N = 40) men, aged 24.4 years, were confronted twice with the Trier Social Stress Test and provided 5 salivary cortisol samples for each of the two testing sessions. A multilevel mixed-effects approach was used to model the cortisol curve throughout the two-day procedure. Habituation to repeated stress was conceptualized as the decrease in the total cortisol levels as well as the change in the cortisol curvilinearity between the first and the second testing session.

Results: Gay participants were characterized by significantly higher cortisol levels throughout both laboratory visits. Their cortisol levels were also predicted by perceived rejection from family due to minority sexual identity, and stigma-related vicarious trauma. Although neither group showed habituation defined as the decrease in cortisol level, the shape of the cortisol curve changed between both visits only in the heterosexual participants.

Conclusions: Increased cortisol levels observed in gay men are predicted by minority stressors. Combined with non-habituation, the upregulation of the HPA axis may constitute a physiological pathway linking stigma to adverse health outcomes.

1. Introduction

Sexual minority people are disproportionately affected by health inequalities including greater prevalence of mental health problems such as depression and anxiety (Plöderl and Tremblay, 2015; Spittlehouse et al., 2019), and physical health issues such as cancer, asthma or diabetes (Conron et al., 2010; Singer et al., 2020). These health inequalities have been studied within the minority stress framework, which associates adverse health outcomes in minority groups with exposure to unique, chronic and socially based stress resulting from societal stigma (Hatzenbuehler et al., 2009; Meyer, 2003). Minority stress model includes both various stigma processes such as experiences of discrimination or expectations of rejection, and stress-ameliorating factors such as individual resilience or social support (Meyer, 2003). Although minority stress has been studied for almost two decades, the physiological pathways linking stigma with health inequalities remain largely unknown and only a few studies have explored this topic (Flentje et al., 2019; Lick et al., 2013).

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Most of these studies have been focused on investigating the hypothalamic-pituitary-adrenocortical (HPA) axis dysregulation profiles linked to chronic stress (Burton et al., 2014; Hatzenbuehler and McLaughlin, 2014; Juster et al., 2015). Indeed, the HPA axis functioning may be of particular interest here, as it becomes activated especially in the context of social-evaluative threats, which constitute the core of minority stress (Dickerson and Kemeny, 2004; Frisch et al., 2015). Cortisol, the end product of the HPA axis, has widespread physiological effects, including increasing the bloodstream glucose availability and immune system activity suppression (Kudielka et al., 2006a; Rohleder et al., 2012). Although, cortisol response to stress is adaptive, prolonged or repeated activation associated with chronic stress may lead to the HPA axis dysregulation and subsequent pathophysiologic changes (McEwen, 1998). The HPA axis magnitude of stress response may, for instance, become inadequate (indicative of hypoactivity or hyperactivity) triggering compensatory actions in associated systems (Miller et al., 2007). Given that exposure to stigma and prejudice constitutes a source of chronic stress, living in a society prejudiced against sexually diverse populations may contribute to increased activity and output of the HPA axis, especially if the stressor is still present in an environment (Miller et al., 2007). It can also become dysregulated in the context of repeated exposure to stressors of the same type, and characterized by impaired or absent habituation (Manigault et al., 2019; McEwen, 1998).

Rapid habituation of cortisol responses after repeated exposure to similar stressors is one of the key characteristics of the HPA axis (Wüst et al., 2005). Being defined as a progressive decline in the magnitude of cortisol response to homotypic stressor, it can already be observed after second exposure and is thought to be normative (Kudielka et al., 2006b; Manigault et al., 2019). Reduced habituation to repeated stress may be indicative of HPA axis dysregulation and has been linked with symptoms of poor health such as vital exhaustion (Kudielka et al., 2006b; Manigault et al., 2019). It has also been predicted by greater post-stress rumination (Gianferante et al., 2014). The latter phenomenon has been suggested to constitute a cognitive mechanism through which stigma may affect well-being in sexual minority individuals (Hatzenbuehler et al., 2009).

Previous experimental studies on HPA axis reactivity in sexual minority individuals, relied on protocols including single exposure to stress and focused on exploring the magnitude and predictors of cortisol responses (e.g., Burton et al., 2014; Hatzenbuehler and McLaughlin, 2014; Juster et al., 2015). According to these studies the exposure to highly stigmatizing environment towards sexual minorities during adolescence is associated with blunted cortisol responses to stress in adulthood (Hatzenbuehler and McLaughlin, 2014) and greater perceived support from the family members predicts reduced cortisol reactivity to stress in sexual minority young adults (Burton et al., 2014). Another study which compared HPA axis reactivity in sexual minority and heterosexual participants, indicated that sexual minority men showed lower cortisol responses to stress than heterosexual men (Juster et al., 2015). Sexual minority men from the same sample were also characterized by decreased symptoms of depression and lower levels of allostatic load (a cumulative toll on the body) indexed with various neuroendocrine, metabolic and immune biomarkers (Juster et al., 2013). The authors suggested that diminished cortisol responses to stress in sexual minority men most likely represent an adaptive strategy protecting them against chronic overactivation of stress response systems and related health adversities (Juster et al., 2015).

None of these studies, however, have also explored the relationship between actual experiences of sexual minority stigma, other than sexual identity concealment, and cortisol responses to stress (Burton et al., 2014; Hatzenbuehler and McLaughlin, 2014; Juster et al., 2015). The previous studies also share some limitations associated with study design, such as using modified versions of standardized stress induction protocols (Burton et al., 2014; Hatzenbuehler and McLaughlin, 2014; Juster et al., 2015), not including heterosexual participants as a control group for comparisons (Burton et al., 2014; Hatzenbuehler and McLaughlin, 2014) or combining bisexual participants with homosexual participants in the analyses (Burton et al., 2014; Hatzenbuehler and McLaughlin, 2014; Juster et al., 2015). Previous studies also relied on samples recruited from large metropolitan areas in US and Canada characterized by progressive social policies towards sexual minorities (Burton et al., 2014; Hatzenbuehler and McLaughlin, 2014; Juster et al., 2015). The results of these investigations cannot be therefore generalized to samples from other, more conservative sociocultural contexts. Finally, none of the previous studies have explored impaired habituation to repeated stress as a potential mechanism linking stigma exposure with increased disease vulnerability in sexual minority populations.

In this study, we aimed at extending the current knowledge on biological outcomes of minority stress by exploring the HPA axis reactivity in the context of repeated stress in healthy gay and heterosexual men. We also evaluated the effect of sexual minority specific predictors of cortisol responses to stress (i.e., experiences of sexual minority stigma) and other predictors (such as trait and state rumination, depressiveness and individual resilience). Our study participants were also recruited from sociocultural context recognized as conservative towards sexual minorities (Poland).

We hypothesized that gay men (compared to heterosexual men) would show (1) decreased habituation of cortisol responses to repeated homotypic stress, (2) either hypocortisolemic or hypercortisolemic cortisol responses to stress, (3) their cortisol levels to be predictable by perceived exposure to sexual minority stressors.

2. Methods and materials

2.1. Participants

Study participants were recruited via fliers and posters, distributed among students of local universities, as well as through social media and websites of Polish LGBT non-governmental organizations (e.g., Equality Signs Federation, The Campaign Against Homophobia). The invitations to participate were also sent to users of selected social networking apps dedicated to sexual minority men (e.g., PlanetRomeo, Fellow and Grindr). The main inclusion criteria were: age between the 18 and 40 years; the absence of neuropsychiatric, endocrine, or other chronic health issues; body mass index (BMI) within the range of 18–30 kg/m^2; no use of psychoactive drugs, steroid hormones, or other medications that might affect cortisol levels. We also decided to focus only on cisgender gay and heterosexual men to increase the power of statistical analyses and data interpretation.

Out of 108 gay and heterosexual men who were invited to the laboratory, 11 (8 gay and 3 heterosexual) failed to attend the second laboratory session and were excluded from this analysis. Cortisol data from additional 8 participants were lost due to technical problems during laboratory analyses. The final study sample consisted of 49 gay and 40 heterosexual men aged 18–37 (M = 24.4; SD = 3.8) (descriptive statistics can be found in Table 1). See Supplement 1 for information about descriptive statistics for participants excluded from this analysis.

2.2. Study design and stress protocol

All research participants underwent a three-step screening for compliance with the inclusion criteria. Firstly, the participants completed a baseline online questionnaire including information on demographics, resilience, trait rumination, recent life changes, depressiveness, health-related behaviors, and health problems. Men who met health-related inclusion criteria were contacted, and screened again, during a phone interview, for chronic and acute health problems. Finally, upon arrival to the laboratory each participant was interviewed about any acute or chronic health issues, and subjected to basic medical examination by a trained physician.

Before the first visit in the laboratory the participants were also emailed detailed instructions on how to prepare for each laboratory

Table 1
Descriptive statistics according to sexual identity of study participants.

	Heterosexual Men	Gay Men	*p*
n	40	49	
Demographics			
Age (years), median (IQR)	23.0 (4.00)	25.0 (6.00)	.032[a]
College experience (%)	80%	80%	.962
City Size > 100k (%)	80%	82%	.845
Sufficient income[1] (%)	80%	82%	.845
Health and health-related behaviors			
BMI, median (IQR)	23.0 (3.48)	22.9 (2.99)	.473
Smoking (%)	28%	24%	.747
Alcohol intake[2], mean (SD)	4.26 (1.60)	4.73 (1.75)	.190
Self-rated health[3], mean (SD)	4.50 (0.75)	4.12 (0.88)	.035
Depressiveness /CESD-R[4], median (IQR)	14.00 (19.00)	17.00 (16.00)	.336[a]
Stress, rumination and resilience			
Recent life changes /RLCQ[5], median (IQR)	466.9 (412.3)	503.5 (447.8)	.748[a]
Resilience /SPP-25[6], median (IQR)	3.92 (0.58)	3.76 (0.56)	.051[a]
Trait Rumination – social[7], median (IQR)	1.90 (0.90)	2.40 (0.90)	.016[a]
Trait Rumination – oneself[7], median (IQR)	2.30 (1.25)	3.00 (1.40)	.015[a]
State rumination – positive[8], median (IQR)	1.50 (0.91)	1.27 (0.64)	.140[a]
State rumination – negative[8], median (IQR)	1.84 (1.34)	1.87 (1.06)	.386[a]
Minority stress[9]			
DHEQ isolation, median (IQR)	–	1.25 (1.25)	–
DHEQ vigilance, median (IQR)	–	1.67 (1.67)	–
DHEQ family of origin, median (IQR)	–	0.17 (0.67)	–
DHEQ vicarious trauma, median (IQR)	–	1.67 (1.33)	–
DHEQ victimization, median (IQR)	–	.000 (0.00)	–
DHEQ harassment, median (IQR)	–	0.17 (0.67)	–
DHEQ total, median (IQR)	–	0.97 (0.59)	–
Laboratory measurements			
Starting Time day 1, mean (SD)	12 PM (2 h)	12 PM (2 h)	.142[a]
Starting Time day 2, mean (SD)	12 PM (2 h)	12 PM (2 h)	.173[a]
Day 1, T0 cortisol (nmol/L), median (IQR)	11.2 (8.9)	15.3 (14.7)	.005[a]
Day 1, T1 cortisol (nmol/L), median (IQR)	17.8 (23.3)	23.4 (21.1)	.034[a]
Day 1, T2 cortisol (nmol/L), median (IQR)	17.1 (19.8)	21.0 (25.4)	.053[a]
Day 1, T3 cortisol (nmol/L), median (IQR)	13.9 (11.7)	16.0 (11.2)	.043[a]
Day 1, T4 cortisol (nmol/L), median (IQR)	9.6 (7.4)	10.8 (8.6)	.334[a]
Day 2, T0 cortisol (nmol/L), median (IQR)	12.5 (15.7)	17.9 (11.7)	.077[a]
Day 2, T1 cortisol (nmol/L), median (IQR)	16.4 (18.2)	25.1 (21.1)	.071[a]
Day 2, T2 cortisol (nmol/L), median (IQR)	13.8 (16)	22.4 (15.7)	.012[a]
Day 2, T3 cortisol (nmol/L), median (IQR)	11.9 (9.4)	15.5 (12.2)	.021[a]
Day 2, T4 cortisol (nmol/L), median (IQR)	9.4 (6.5)	11.6 (10.5)	.113[a]
Day 1 AUCg[10] index, median (IQR)	1430.2 (1475.8)	1993.6 (1700.4)	.016[a]
Day 2 AUCg index, median (IQR)	1284.5 (1256)	2070.5 (1384.5)	.018[a]
Day 1 AUCi[11] index, median (IQR)	347.5 (742.8)	58.6 (868.6)	.482[a]
Day 2 AUCi index, median (IQR)	18.7 (830.3)	-84.0 (995.4)	.886[a]

Note: [1]Dichotomized version of a question "Is it difficult for you to make ends meet". Responses "with great difficulty", "with difficulty" were coded as 0. Responses "minor difficulties" and "easily" where coded as 1; [2] Frequency of alcohol drinking rated on a scale from 0 (never) to 9 (everyday), [3]Six-point scale ranging from 1 (very bad) to 6 (very good); [4]The Center for Epidemiologic Studies Depression Scale - Revised; [5]Recent Life Changes Questionnaire; [6]Resilience Measurement Scale SPP-25; [7]Rumination Questionnaire with social and oneself subscales; [8]Thoughts Questionnaire with positive and negative ruminations subscales; [9]The Daily Heterosexist Experiences Questionnaire subscales; [10] Area Under the Curve relative to the ground (AUCg) index; [11] Area Under the Curve relative to increase index (AUCi); [a]p value from Mann–Whitney *U* test.

session. We instructed the participants to avoid intensive physical exercises and drinking alcohol the day before, and on the day of each session, as well as to avoid caffeinated drinks or brushing teeth within two hours before each appointment. Each participant was also interviewed in the laboratory about the compliance with the instructions provided.

All laboratory sessions were held on weekends, and were scheduled between 10:00 a.m. and 5:00 p.m. The eligible participants were subjected to standardized stress protocol twice—at identical times on two consecutive days.

Upon arrival to the laboratory, the study procedure was described to the participants in detail, and a written consent was obtained. Next, the participants were examined by a physician who also performed anthropological measurements. Body height was measured with portable stadiometer in a standardized position, and body weight with BC-418 Segmental Body Composition Analyzer Tanita. The participants were then seated in a testing room, and the stress protocol was initiated on average within 30 min from arrival.

We used the Trier Social Stress Test (TSST) which is a standardized laboratory protocol to elicit the HPA axis responses (Kirschbaum et al., 1993). The TSST involved a 5-min preparation period followed by a 5-min speech task (mock job interview), and a 5-min arithmetic task (serial subtraction) performed in front of an expert panel comprising two research assistants, a man and a woman, trained to maintain neutral facial expressions throughout the protocol. Participants were instructed to take over the role of an applicant for their dream job and convince their audience that they are the perfect candidate for this position (Kudielka et al., 2007). The stress protocol also included information that participants' performance will be recorded and later analyzed by communication skills experts. To support this instruction, the participants were positioned in a designated place in front of a camera for both the speech and arithmetic tasks (Kudielka et al., 2007). Participants' sexual identity remained undisclosed to all research assistants except for the research group supervisor.

To avoid the effect of learning and familiarity with the experts, we replaced the members of the expert panel and slightly modified the second day's TSST instructions (Petrowski et al., 2012). The participants were informed that the expert panel would focus on verbal aspects of their presentation during the first day, and on non-verbal aspects during the second session. Similarly, on the first day participants were instructed to count backwards from 1022 in steps of 13, while on the second day the steps equaled 17. Having completed the TSST the participants returned to the testing room, and followed the study protocol which included saliva sampling at specific time intervals, and filling additional questionnaires.

Following the first visit, the experimenter made sure during a brief conversation that the participants were not distressed anymore, thanked for their participation in the study and confirmed the time of the second session. The participants were also assured in a neutral manner that they performed well in front of the expert panel given the circumstances (Morris and Rao, 2014). If a participant expressed clear intent not to repeat the procedure on the next day, he was debriefed immediately. Otherwise debriefing occurred after the second TSST and included information about the nature of the stressor, as well as explanation that the participants' performance was not recorded (Kudielka et al., 2007). The participants were reimbursed for their time and travel costs with 60 PLN (approximately 15 USD) per session. The study was approved by the Bioethics Committee of Jagiellonian University.

2.3. Endocrine measures

The HPA axis responses to stress were determined by measuring

cortisol concentrations in saliva samples (1–1.5 mL each) collected using the passive drool method. The first saliva sample (T0) was collected 5 min before the TSST procedure. The subsequent saliva samples were collected 25 (T1), 35 (T2), 55 (T3) and 95 (T4) minutes after the onset of the TSST. The samples were instantly frozen, and stored for analyses in the temperature of − 20 °C. All measurements were conducted in duplicate, using commercially available immunoenzymatic Cortisol Saliva kits (DiaMetra, Italy; sensitivity: 0.12 ng/mL; standard range: 0.5–100 ng/mL). The intra- and inter-assay CVs were below 10% and 15%, respectively. Cortisol concentrations are expressed as nmol/L.

2.4. Psychological assessments

We collected demographic information about age, education, place of residence, and current financial situation of study participants. The participants were assigned to study groups based on their self-reported sexual identity during the initial assessment (available options included: *heterosexual*; *gay*; *bisexual*; *queer*; *unlabeled* and *other, please specify*).

We used several questionnaires to capture factors possibly related to cortisol responses to acute stress. Polish adaptation of *The Center for Epidemiologic Studies Depression Scale – Revised (CESD-R)* (Eaton et al., 2004; Koziara, 2016) was used to control for any variation of the HPA axis reactivity related to the level of depression in the studied sample. CESD-R consists of 20 items describing various symptoms of depression. Participants were instructed to indicate on a 5-item scale how often they felt in a described way during last 2 weeks (0 = *not at all or less than one day* to 4 = *nearly every day for 2 weeks*). Polish adaptation of this questionnaire is characterized by good psychometric properties (Koziara, 2016). The CESD-R sum score was used in the analysis.

The exposure to major life stressors was measured with the Polish adaptation of *Recent Life Changes Questionnaire (RLCQ)* (Rahe, 1975; Sobolewski et al., 1999). The questionnaire is composed of 75 items—life events from five major life domains such as health, work, family, finances, and social life. We assigned numerical value to each life event, indicating how stressful this event was based on Polish stressfulness rating and used sum score in the analysis (Sobolewski et al., 1999).

Individual resilience, defined as an ability to deal with various life stressors, was captured with *Resilience Measurement Scale SPP-25* (Ogińska-Bulik and Juczyński, 2008). This questionnaire consists of 25 statements, each rated on 5-point scale with regard to how well it describes the participant. Higher average score indicates greater resilience to stress.

To account for individual tendency to experience unwanted and recurrent thoughts on past events, *Trait Rumination Questionnaire* was used (Baryła and Wojciszke, 2005). This Polish questionnaire consists of 20 items—10 items related to ruminations associated with social world (e.g. *It hurts me that some people got something they didn't deserve*) and 10 items comprising ruminations about oneself (e.g. *I blame myself for inappropriate behavior in the past*). The participants rated each statement on a 5-point scale ranging from 1 = *never* to 5 = *very often*. We included an average score for each factor (*Trait Rumination – social world* and *Trait Rumination – oneself*) in the analysis. Higher score indicates greater tendency to ruminate.

In addition, we collected data on smoking (dichotomized to indicate current smokers vs non-smokers regardless of smoking frequency), and alcohol intake (rated on a scale from 0 = *I don't drink alcohol* to 9 = *I drink every day*). A single-item 6-point scale ranging from 1 = *very bad* to 6 = *very good* was used as a brief indicator of an overall, self-rated physical health.

Post stress, state rumination was measured during the second visit using *Thoughts Questionnaire* (Edwards et al., 2003; Gianferante et al., 2014), which was designed to measure both the negative and the positive post-event ruminations. The questionnaire consists of 29 items rated on a 5-point scale ranging from 0 = *never* to 4 = *very often*. Before the second TSST, the participants were instructed to indicate how often, since their first TSST, they had experienced both negative (e.g., *I made a fool of myself*) and positive (e.g., *I came across as self-assured*) thoughts about their performance in front of the expert panel. Given that there is some evidence in the literature that positive ruminations are characterized by distinct psychological dynamics from negative ruminations (Gilbert et al., 2017) we calculated average for both indexes separately and included both in the analysis.

The gay participants were additionally administered *The Daily Heterosexist Experiences Questionnaire (DHEQ)* (Balsam et al., 2013; Polish adaptation by Mijas and Koziara, 2020) designed to assess the exposure to sexual minority stressors. Six DHEQ factors were included in this study: 'Victimization' capturing the experiences of physical violence; 'Harassment' describing the experiences of discrimination and verbal abuse; 'Family of Origin' depicting the rejection by parents and siblings; 'Vicarious Trauma' comprising the feelings of distress related to learning about the discrimination experienced by LGBT people; 'Vigilance' capturing efforts made to conceal one's sexual identity, and 'Isolation' describing the feelings of alienation associated with being an LGBT person. Each item is rated on 6-point scale with 0 = *did not happen/not applicable to me*, 1 = *it happened, and it bothered me NOT AT ALL*, 2 = *it happened, and it bothered me A LITTLE BIT*, 3 = *it happened, and it bothered me MODERATELY*, 4 = *it happened, and it bothered me QUITE A BIT* and 5 = *it happened, and it bothered me EXTREMELY*. Higher average scores indicate greater perceived exposure to sexual minority stigma.

Alpha coefficients for each questionnaire can be found in Supplement 1, Table S5.

2.5. Data analysis

The data analysis was performed using STATA 14 software (StataCorp, 2015). The multilevel models were estimated using STATA's xtmixed command under REML.

2.5.1. Preliminary analyses

To compare the heterosexual and gay participants with respect to demographics and other potential predictors of cortisol response to stress, *t*-tests, Mann-Whitney *U* tests or Chi-squared tests were performed (Table 1).

2.5.2. Analysis of habituation of cortisol responses to homotypic stress

The cortisol data were naturally log-transformed to reduce skewness, and then centered on the first sample of the first session (day 1).

To test primary hypothesis, a multilevel mixed-effects approach was used to model the cortisol curve throughout the two-day procedure. Following Manigault et al. (2019) we conceptualized habituation as both the change in the shape of the cortisol curve from the first to the second session (reflected by the three-way interaction of sexual identity by timepoint by day in the three-level model) and the change in the total cortisol level between the first and the second session (reflected by the two-way interaction of sexual identity by day in the two-level model). We used this approach because it allows to better control the interindividual variability in cortisol responses to stress. Additionally, two widely used cortisol indexes, Area Under the Curve relative to the ground (AUCg) and Area Under the Curve relative to increase (AUCi) were computed using the trapezoid formula (Pruessner et al., 2003) to facilitate the comparison of our results with the previous literature.

In the three-level model the cortisol concentrations for each saliva sample (level-1) were nested within each of the two testing sessions (level-2) which, in turn, were nested within each participant (level-3). This allowed to analyze cortisol response as a curve, modeled in time, while controlling for within-person and within-day variance. Timepoints in the three-level model were defined as minutes since the collection of the first saliva sample (T0) and each consecutive sample: T1 (T0 + 30 min), T2 (T0 + 40 min), T3 (T0 + 60 min), T4 (T0 + 100 min). The timepoints were modeled both linearly and

quadratically to represent the dynamics of cortisol response. Two-way interaction terms were the products of sexual identity (heterosexual = 0, gay = 1) by timepoint (T0 – T4) defined both linearly and quadratically, as well as sexual identity (heterosexual = 0, gay = 1) by testing day (day 1 = 0, day 2 = 1). The three-way interaction terms were defined as sexual identity by linear timepoint by testing day, and sexual identity by quadratic timepoint by testing day.

First, the initial model (Model 0) was constructed, which included sexual identity as predictor (heterosexual = 0, gay = 1), all two-way and three-way interaction effects and five core covariates: BMI index, age, smoking status (non-smoking = 0, smoking = 1), protocol starting time (between 10.00 AM and 5.00 PM) and self-rated health (on a 6-point Likert scale). Subsequent three models (Models 1–3) separately tested potential predictors of cortisol responses to stress, including: the level of depression, resilience, recent life changes, trait and state ruminations. The final three-level model (Model 4) comprehensively tested for all potential predictors of the differences in cortisol responses for gay and heterosexual men. All predictors in models were grand mean centered. Both preliminary models and the final model are included in Supplement 1, Table S2. The final model is also included in the Table 2.

In the two-level model, total cortisol concentration was represented by the mean cortisol for five saliva samples separately for each day (level-1) nested in each participant (level-2). The construction of the final two-level model followed similar procedure as in the case of three-level model. First, the baseline model (Model 0) which included sexual identity as predictor (heterosexual = 0, gay = 1), two-way interaction of sexual identity (heterosexual = 0, gay = 1) by day (day 1 = 0, day 2 = 1), and five core covariates: BMI index, age, smoking status, protocol starting time and self-rated health was constructed. Three subsequent models (Model 1–3) tested separately for all potential predictors of cortisol responses. The final model (Model 4), comprehensively tested for all predictors. All models (Model 0–4) are included in the Supplement 1, Table S3.

2.5.3. Analysis of minority stress predictors of cortisol response to stress

Additionally, a two-level model was created to investigate the associations between the exposure to minority stress processes and cortisol levels in gay participants. Given that we observed the most consistent differences between heterosexual and gay participants with respect to the total cortisol output tested by the two-level model, we decided to continue this approach and examine the minority stress predictors of total cortisol levels in gay participants. The construction of the final model for gay participants followed a similar procedure. The baseline model (Model 0) included five core covariates (BMI index, age, smoking status, protocol starting time and self-rated health) and analyzed mean cortisol separately for each day (level-1) as nested in each participant (level-2). The subsequent seven models (Model 1–7) additionally included all potential predictors of cortisol responses (the level of depression, resilience, recent life changes, trait and state ruminations) and separately tested for each of 6 included DHEQ factors capturing various sexual minority stressors, as well as average DHEQ score. The final two models tested for all DHEQ factors (Model 8) and additionally included two-way interactions of each DHEQ factor which consistently and significantly predicted cortisol output in gay men by day of the testing session (Model 9). All models are included in the Supplement 1, Table S4. The final model is displayed in the Table 2.

3. Results

3.1. Preliminary analyses

Compared to heterosexual participants, gay men were significantly older (Med_{gay} = 25, $Med_{heterosexual}$ = 23, p = .032), and on average assessed their health as worse than the heterosexual men (M_{gay} = 4.12, $M_{heterosexual}$ = 4.50, p = .035) (Table 1). Another significant difference was related to the individual tendency to ruminate about social

Table 2
Multilevel mixed regression model of cortisol (log) responses to TSST protocol in a sample of gay and heterosexual men (n = 89) and in a subsample of gay men alone (n = 49).

Final Model (Gay vs Heterosexual men)				**Final Model** (Gay men)			
	b	**s.e.**	**p**		**b**	**s.e.**	**p**
BMI	0.00	0.02	.930	BMI	-0.02	0.02	.443
Age	-0.03	0.02	.108	Age	-0.04	0.01	.010
Smoking	0.23	0.14	.110	Smoking	0.29	0.13	.024
Starting time	0.05	0.03	.189	Starting time	0.16	0.04	< 0.001
Self-rated health	-0.09	0.08	.233	Self-rated health	-0.06	0.07	.420
SPP-25	-0.17	0.13	.194	Resilience /SPP-25	0.14	0.12	.233
RLCQ	0.00	0.00	.827	Recent life changes /RLCQ	-0.00	0.00	.034
CESD-R	-0.01	0.01	.379	Depressiveness /CESD-R	-0.00	0.01	.994
TR social	0.02	0.08	.778	TR social	0.12	0.09	.173
TR oneself	0.00	0.08	.962	TR oneself	-0.06	0.08	.412
SR positive	0.04	0.06	.464	SR positive	0.06	0.11	.612
SR negative	-0.05	0.04	.226	SR negative	0.18	0.10	.090
Gay men	0.43	0.15	.003	DHEQ isolation	0.11	0.07	.127
timepoint	0.01	0.00	< 0.001	DHEQ vigilance	-0.00	0.06	.995
$timepoint^2$	-0.00	0.00	< 0.001	DHEQ family of origin	.34	0.12	.004
				DHEQ vicarious trauma	-0.20	0.09	.031
Gay#timepoint	-0.00	0.00	.810	DHEQ victimization	0.15	0.16	.362
$Gay\#timepoint^2$	-0.00	0.00	.625	DHEQ harassment	-0.07	0.14	.599
Gay#day				**DHEQ family#day = 2**	-0.11	0.11	.334
Heterosexual#day2	0.22	0.08	.005	**DHEQ vicar#day = 2**	-0.04	0.14	.766
Gay#day1	-0.06	0.07	.427				
				intercept	0.10	0.07	.163
Gay#day#timepoint							
Heterosexual#day2	-0.01	0.00	.006				
Gay#day2	-0.00	0.00	.116				
$Gay\#day\#timepoint^2$							
Heterosexual#day2	0.00	0.00	.059				
Gay#day2	0.00	0.00	.107				
intercept	-0.32	0.11	.004				

Final model gay vs heterosexual men: chi^2 (23) = 284.3, $p < .001$, AIC = 1313.2, BIC = 1471.3. Final model gay men: chi^2 (20) = 56.3, $p < .001$, AIC = 247.8, BIC = 307.3

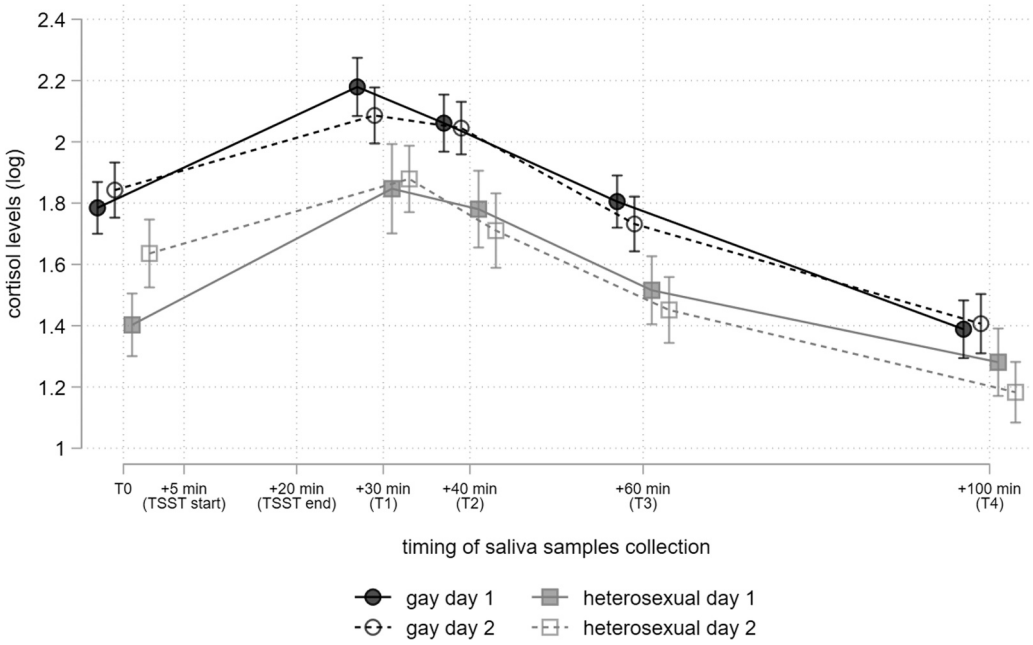

Fig. 1. Observed mean cortisol levels as a function of testing session (day 1 vs day 2) and study group (gay men vs heterosexual men). Cortisol data was centered and log-transformed to reduce skewness.

environment (Med_{gay} = 2.40, $Med_{heterosexual}$ = 1.90, p = .016) and oneself (Med_{gay} = 3.00, $Med_{heterosexual}$ = 2.20, p = .015).

During the first session, gay men displayed significantly higher cortisol levels 5 min before the TSST (T0), 25 min (T1) and 55 min (T3) after the initiation of the stress protocol (TSST). On the second day, gay men were characterized by significantly greater cortisol concentrations 35 min (T2) and 55 min (T3) after the initiation of TSST. The pattern of higher cortisol levels in gay men as compared to heterosexual men was maintained throughout both sessions (Fig. 1). Total cortisol output as reflected by the AUCg index was significantly greater in the case of gay men during both the first (Med_{gay} = 1993.6, $Med_{heterosexual}$ = 1430.2, p = .016) and the second (Med_{gay} = 2070.5, $Med_{heterosexual}$ = 1284.5, p = .018) testing day.

3.2. Comparison of habituation in gay and heterosexual participants

The results of mixed regression models of cortisol responses to the TSST are presented in Table 2. Gay participants were characterized by significantly higher initial (T0) cortisol level on the first day of the procedure as compared to heterosexual men (b = 0.43, p = .003). The timepoint variable and its quadratic term were statistically significant predictors of the cortisol concentrations (timepoint: b = 0.01, p < .001, $timepoint_{quadratic}$: b = −0.00, p < .001), indicating that they changed during each visit in a nonlinear way.

The two-way interaction between sexual identity and the day of the procedure was significant in the case of heterosexual participants (b = 0.22, p = .005), indicating that the initial (T0) cortisol level was significantly increased on the second testing day compared to the first testing session in this group. No such difference was observed in gay men (b = −0.06, p = .427).

Similarly, the three-way interaction between sexual identity, the day of the procedure and timepoint was significant only in the case of heterosexual participants (b = −0.01, p = .006) indicating that the linear slope corresponding to T0 changed from the first to the second testing session in this group. The three-way interaction of the day of the procedure, the quadratic timepoint, and the sexual identity, approached statistical significance in heterosexual men (b = 0.00, p = .059). In the case of gay participants neither the three-way interaction of sexual identity by timepoint by day (b = −0.00, p = .116), nor the interaction of sexual identity by quadratic timepoint by day (b = 0.00, p = .107) reached statistical significance.

Total cortisol output was tested in two-level model (see Table S3 in Supplement 1) in which we detected higher overall cortisol levels in gay men compared to heterosexual men on both testing sessions (margins for both days: b = 0.29, p = .038). When we calculated the difference for each day separately, we obtained statistically significant results on day one (b = 0.30, p = .037), and marginally significant results on the day two (b =0.28, p = .054). The change in the overall cortisol output between the first and the second testing session was not significant in either group (b = 0.00, p = .947 in the case of heterosexual men; b = −0.02, p = .698 in the case of gay men).

Trait and state rumination, depressiveness, recent life changes and resilience did not significantly predict cortisol levels in either of the groups (Table 2).

3.3. Minority stress predictors of cortisol responses to stress

In the model for gay men, the cortisol levels were predicted by age – indicating that older participants displayed decreased total cortisol (b = −0.04, p = .010), starting time of the procedure with later time predicting higher cortisol concentrations (b = 0.16, p < .001), and smoking status (b = 0.29, p = .024) with smoking participants characterized by higher cortisol levels.

Among the factors of DHEQ questionnaire 'Family of origin' (b = 0.34, p = .004) and 'Vicarious trauma' (b = −0.20, p = .031) significantly predicted cortisol levels in gay men on the first day of laboratory observation. The greater the perceived stress associated with rejection from family was, the higher the total cortisol concentrations were. In the case of 'Vicarious trauma' this relationship was negative. No significant interactions of both factors and day of the procedure were observed, indicating that both DHEQ factors worked in the same way at day one and day two of the procedure.

None of the other predictors included in the model—such as state and trait rumination, depressiveness, or resilience—predicted cortisol levels in gay participants (Table 2).

4. Discussion

The aim of our study was to extend the current knowledge on biological outcomes of minority stress by exploring the HPA axis reactivity in gay and heterosexual participants in the context of repeated stress. Specifically, we investigated whether gay men show cortisol response profiles indicative of HPA axis dysregulation, including impaired habituation to repeated homotypic stress (Manigault et al., 2019; McEwen, 1998). As hypothesized, we observed significant differences between gay and heterosexual participants related to the magnitude of the cortisol responses to stress. Our results also suggest that sexual identity may be associated with the HPA axis ability to habituate to repeated homotypic stress. Additionally, we showed that experiences of sexual minority stigma were associated with cortisol levels in gay men.

Our results reveal that gay men are characterized by higher cortisol levels as compared to heterosexual men. This remains significant even when controlling for potential confounders such as depressiveness or recent life changes. Although we observed increased tendency to ruminate about both the social world and oneself in gay men, neither state nor trait rumination significantly predicted cortisol concentrations in the study sample.

The direction of observed differences in cortisol reactivity between gay and heterosexual men was contrary to previous research which indicated that sexual minority men showed diminished cortisol response to acute stress and were characterized by lower levels of allostatic load compared to heterosexual participants (Juster et al., 2015). On the other hand, observational studies on cortisol diurnal profiles in minority samples demonstrated that exposure to stigma was associated with elevated cortisol levels (DuBois et al., 2017; Parra et al., 2016). For example, according to study by Parra et al. (2016) exposure to various minority stressors was associated with elevated diurnal cortisol levels among sexual minority young adults. Another study conducted in transgender men indicated that greater exposure to stigma related to transitioning was related to higher diurnal cortisol (DuBois et al., 2017). Consistently, Manigault et al. (2018b) observed increased cortisol output in sexual minority young adults characterized by low family disclosure. It is possible, therefore, that the observed differences between gay and heterosexual participants in our sample might be primarily driven by elevated diurnal cortisol profiles in gay men and not the differences in cortisol reactivity to acute stress.

Pathogenic changes of the HPA axis reactivity associated with chronic stress can be characterized both by up- and downregulation depending on the time that elapsed since the stressor onset (Miller et al., 2007). Initially, following adverse life events, the HPA axis is characterized by hyperactivation which, over time, rebounds below normal (Miller et al., 2007). Our study participants were mainly young adults ($M = 24.4$ years) and given that younger sexual minority persons are more exposed to sexual minority stressors, among them burden associated with the coming out (Bruce et al., 2015; Russell and Fish, 2016), it is justified to expect that gay men in our sample will still show elevated cortisol levels. This interpretation is also consistent with the fact that older age among gay participants predicted decreased cortisol levels.

Therefore, it is possible that the differences between Juster et al. (2015), who observed diminished cortisol responses to acute stress in sexual minority men, and our research can be attributed to the differences in actual exposure to sexual minority stigma in studied populations. Aforementioned study (Juster et al., 2015) was conducted in Canada (Montreal) which has some of the most progressive policies on sexual minorities. Our research was conducted in Poland, which has recently been rated by ILGA-Europe (ILGA-Europe, 2020) as a country with the worst human rights situation of LGBT people in the European Union. This includes both lack of legal protection from hate speech or hate crimes, and no legal recognition of same-sex relationships or adoption rights for same-sex couples. Nearly 70% of LGBT participants in a study conducted by The Campaign Against Homophobia revealed being exposed to at least one type of violence (including verbal abuse, threats, or physical violence) due to their gender or sexual identity within two years preceding the data collection (Świder et al., 2017). According to the same study only 25% of mothers and 12% of fathers fully accepted sexual or gender identity of their LGBT children (Świder et al., 2017).

In our study, the cortisol concentrations among gay participants were also associated with two dimensions of sexual minority stigma: the perceived rejection from family members due to minority sexuality, and the vicarious trauma defined as feelings of distress associated with learning about discrimination experienced by other LGBT individuals. Greater perceived stigma associated with family rejection predicted higher cortisol levels, and greater vicarious trauma was associated with diminished total cortisol. This difference may be explained by differences in core emotions elicited by each stressor (Miller et al., 2007). Being rejected by family due to sexual identity may be associated with feeling of shame, and may constitute a particularly severe stressor in Poland—a country where traditional family is held in high regard. According to the meta-analysis by Miller et al. (2007) chronic stressors, which are likely to involve feelings of shame, lead to upregulation of the HPA axis. This result is also consistent with studies demonstrating that shame mediates the relationship between stigma and distress (Mereish and Poteat, 2015), and that family rejection predicts poorer health outcomes in LGB samples (Ryan et al., 2009). Moreover, learning about other LGBT persons being discriminated will most likely evoke increased anxiety. Consistently with previous studies, which associated anxiety symptoms with blunted cortisol responses to acute stress (Brooks and Robles, 2009; Fiksdal et al., 2019), vicarious trauma negatively predicted total cortisol levels in gay men.

Contrary to our hypothesis we did not observe the habituation defined as the change in the cortisol levels in neither of the groups of participants. We expected to see this pattern in the control group of heterosexual participants. The habituation of the HPA axis responses to repeated homotypic stressor is determined by several mediators, including stress modality and intensity (Kirschbaum et al., 1995; Wüst et al., 2005). Animal studies indicated that stimulus intensity is inversely related to the magnitude of habituation (Pitman et al., 1990). It is possible that the changes in the TSST protocol during the second day of laboratory observation, introduced to avoid learning and repetition effect, maintained the perceived stressor novelty, which is one of the driving factors of cortisol responses to stress. However, Petrowski et al. (2012) introduced similar changes in the TSST protocol during the second session, including changing the instruction for the arithmetic task and changing the evaluative panel, and still observed habituation over two consecutive days. Additionally, similar lack of habituation was observed also by Manigault et al. (2019) in young, healthy men. The authors attributed this effect to higher exposure to stress in studied group (Manigault et al., 2019). The range of habituation to repeated stress of the same type is also characterized by significant inter-individual variance, which suggests that some people simply need more time to habituate (Wüst et al., 2005). Therefore, more exposures to homotypic stress may be necessary to observe the pattern of total cortisol decrease and to capture potential differences in habituation defined as the change in total cortisol levels between gay and heterosexual men (Allen et al., 2014).

Although, while analyzing changes in the shape of the cortisol curve between both testing sessions we observed an interesting dynamics of cortisol reactivity to repeated stress in heterosexual men, this result should be interpreted with caution. The three-way interaction of sexual identity by day by quadratic timepoint, indicative of changes in the cortisol curve, only approached statistical significance ($p = .059$). This effect was also most likely driven by significantly increased baseline cortisol levels in heterosexual participants during the second testing session and associated with significant three-way interaction of sexual identity by day by timepoint defined in a linear way. The latter interaction means that the linear slopes corresponding to the baseline cortisol and best fitted to cortisol responses during each testing session changed

from the first to the second visit in heterosexual participants. We observed no such effects in gay men. One can speculate that greater pre-stress cortisol may be indicative of greater active coping (Manigault et al., 2018a, 2019). It can be also interpreted as indicative of increased anticipatory stress or even sensitization of cortisol response, however, no associated change in the total cortisol output rather contradicts the latter suggestion. Future studies should further explore the changes in cortisol curvilinearity in the context of repeated stress, preferably using designs with more repeated exposures and more pre-stress cortisol measurements.

Interestingly, in the case of gay men we observed no such dynamics – neither habituation defined as a change in total cortisol levels, nor as any change in cortisol curvilinearity between both testing sessions emerged in this group. Combined with elevated cortisol responses to stress, reduced habituation may contribute to greater overall exposure to stress mediators, associated wear-and-tear of the body (i.e., allostatic load) and subsequent greater susceptibility to disease in this population (McEwen, 1998). The inability to habituate to homotypic stress has been interpreted in the literature as one of the scenarios leading to greater cumulative consequences of stress (Kudielka et al., 2006b; McEwen, 1998). This result should be however interpreted with caution given that we did not observe the habituation defined as the change in the total cortisol level in a control group of heterosexual participants as well.

There are several limitations of our study. Firstly, we slightly modified the TSST protocol during the second testing day (by replacing the members of the expert panel and by changing the instruction for the arithmetic task during the second session), which might have affected our results concerning habituation of cortisol responses to repeated stress. Secondly, we focused only on gay-identified men, thus our results cannot be generalized to individuals with other minority sexual identities. Thirdly, we only controlled for the starting time of the stress protocol in the analysis instead of the wake time of participants which could potentially influence pre-stressor cortisol levels in study participants (Manigault et al., 2019). We also used a single biomarker of the HPA axis reactivity which offers limited insight into the mechanisms guarding its regulation. Finally, the design of our study did not allow to determine the causality between perceived stigma and cortisol stress responses.

Our results, however, significantly extend the literature on biological outcomes of minority stress and have important practical implications concerning potential mechanisms linking minority stress with health inequalities. We demonstrated novel findings indicating that cortisol responses to stress in gay men recruited from conservative sociocultural context are significantly elevated compared to heterosexual men. We also showed associations between HPA axis reactivity and exposure to minority stressors in gay participants.

5. Conclusions

Our study was the first to investigate the range of habituation to repeated homotypic stress in gay men and compare the HPA axis reactivity to repeated stress exposure between heterosexual and gay participants. It has been suggested that the dysregulation of the HPA axis constitutes a possible pathway linking minority stress with health disparities between sexual minority and general populations (Lick et al., 2013). Both greater hyperactive cortisol responses and failure to habituate observed in gay men may lead to overexposure to stress hormones, and thus to increased disease vulnerability. Future studies should further investigate the significance of the observed effects in producing health disparities in sexual minority populations.

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CRediT authorship contribution statement

MM and GJ: were responsible for study design; **MM, MB, MP, KaKo, AG and KrzKa**: were responsible for data collection; **MM, MB, KrzKa and KaKo**: were responsible for statistical analyses and interpretation of data. All authors participated in manuscript creation and gave final approval for publication.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2021.105325.

References

Allen, A.P., Kennedy, P.J., Cryan, J.F., Dinan, T.G., Clarke, G., 2014. Biological and psychological markers of stress in humans: focus on the trier social stress test. Neurosci. Biobehav. Rev. 38, 94–124.

Balsam, K.F., Beadnell, B., Molina, Y., 2013. The daily heterosexist experiences questionnaire: measuring minority stress among lesbian, gay, bisexual, and transgender adults. Meas. Eval. Couns. Dev. 46, 3–25.

Baryła, W., Wojciszke, B., 2005. Kwestionariusz ruminacji. Stud. Psychol. 4, 5–22.

Brooks, K.P., Robles, T.F., 2009. Recent depressive and anxious symptoms predict cortisol responses to stress in men. Psychoneuroendocrinology 34, 1041–1049.

Bruce, D., Harper, G.W., Bauermeister, J.A., 2015. Minority stress, positive identity development, and depressive symptoms: Implications for resilience among sexual minority male youth. Psychol. Sex. Orientat. Gend. Divers. 2, 287–296.

Burton, C.L., Bonanno, G.A., Hatzenbuehler, M.L., 2014. Familial social support predicts a reduced cortisol response to stress in sexual minority young adults. Psychoneuroendocrinology 47, 241–245.

Conron, K.J., Mimiaga, M.J., Landers, S.J., 2010. A population-based study of sexual orientation identity and gender differences in adult health. Am. J. Public Health 100, 1953–1960.

Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol. Bull. 130, 355–391.

DuBois, L.Z., Powers, S., Everett, B.G., Juster, R.-P., 2017. Stigma and diurnal cortisol among transitioning transgender men. Psychoneuroendocrinology 82, 59–66.

Eaton, W.W., Smith, C., Ybarra, M., Muntaner, C., Tien, A., 2004. Center for epidemiologic studies depression scale: review and revision (CESD and CESD-R). In: Maruish, M.E. (Ed.), The Use of Psychological Testing for Treatment Planning and Outcomes Assessment: Instruments for Adults. Lawrence Erlbaum Associates Publishers, pp. 363–377.

Edwards, S.L., Rapee, R.M., Franklin, J., 2003. Postevent rumination and recall bias for a social performance event in high and low socially anxious individuals. Cogn. Ther. Res. 27, 603–617.

ILGA-Europe, 2020. Country Ranking. Rainbow Europe. Retrieved from ⟨https://ilga-europe.org/rainboweurope/2020⟩ (Accessed 6 June 2020).

Fiksdal, A., Hanlin, L., Kuras, Y., Gianferante, D., Chen, X., Thoma, M.V., Rohleder, N., 2019. Associations between symptoms of depression and anxiety and cortisol responses to and recovery from acute stress. Psychoneuroendocrinology 102, 44–52.

Flentje, A., Heck, N.C., Brennan, J.M., Meyer, I.H., 2019. The relationship between minority stress and biological outcomes: a systematic review. J. Behav. Med. 1–22.

Frisch, J.U., Häusser, J.A., Mojzisch, A., 2015. The trier social stress test as a paradigm to study how people respond to threat in social interactions. Front. Psychol. 6, 14.

Gianferante, D., Thoma, M.V., Hanlin, L., Chen, X., Breines, J.G., Zoccola, P.M., Rohleder, N., 2014. Post-stress rumination predicts HPA axis responses to repeated acute stress. Psychoneuroendocrinology 49, 244–252.

Gilbert, K., Luking, K., Pagliaccio, D., Luby, J., Barch, D., 2017. Dampening, positive rumination, and positive life events: associations with depressive symptoms in children at risk for depression. Cogn. Ther. Res. 41, 31–42.

Hatzenbuehler, M.L., McLaughlin, K.A., 2014. Structural stigma and hypothalamic–pituitary–adrenocortical axis reactivity in lesbian, gay, and bisexual young adults. Ann. Behav. Med. 47, 39–47.

Hatzenbuehler, M.L., Nolen-Hoeksema, S., Dovidio, J., 2009. How does stigma "get under the skin"? The mediating role of emotion regulation. Psychol. Sci. 20, 1282–1289.
Juster, R.-P., Hatzenbuehler, M.L., Mendrek, A., Pfaus, J.G., Smith, N.G., Johnson, P.J., Lefebvre-Louis, J.-P., Raymond, C., Marin, M.-F., Sindi, S., 2015. Sexual orientation modulates endocrine stress reactivity. Biol. Psychiatry 77, 668–676.
Juster, R.-P., Smith, N.G., Ouellet, É., Sindi, S., Lupien, S.J., 2013. Sexual orientation and disclosure in relation to psychiatric symptoms, diurnal cortisol, and allostatic load. Psychosom. Med. 75, 103–116.
Kirschbaum, C., Pirke, K.-M., Hellhammer, D.H., 1993. The 'Trier Social Stress Test'–a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28, 76–81.
Kirschbaum, C., Prussner, J.C., Stone, A.A., Federenko, I., Gaab, J., Lintz, D., Schommer, N., Hellhammer, D.H., 1995. Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy men. Psychosom. Med. 57, 468–474.
Koziara, K., 2016. Assessment of depressiveness in population. Psychometric evaluation of the Polish version of the CESD-R. Psychiatr. Pol. 50, 1109–1117.
Kudielka, B.M., Bellingrath, S., Hellhammer, D.H., 2006a. Cortisol in burnout and vital exhaustion: an overview. G Ital. Med Lav. Erg. 28, 34–42.
Kudielka, B.M., von Känel, R., Preckel, D., Zgraggen, L., Mischler, K., Fischer, J.E., 2006b. Exhaustion is associated with reduced habituation of free cortisol responses to repeated acute psychosocial stress. Biol. Psychol. 72, 147–153.
Kudielka, B.M., Hellhammer, D.H., Kirschbaum, C., 2007. Ten years of research with the trier social stress test–revisited. In: Harmon-Jones, E., Winkelman, P. (Eds.), Social Neuroscience: Integrating Biological and Psychological Explanations of Social Behavior. The Guilford Press, New York, pp. 56–83.
Lick, D.J., Durso, L.E., Johnson, K.L., 2013. Minority stress and physical health among sexual minorities. Perspect. Psychol. Sci. 8, 521–548.
Manigault, A.W., Shorey, R.C., Hamilton, K., Scanlin, M.C., Woody, A., Figueroa, W.S., France, C.R., Zoccola, P.M., 2019. Cognitive behavioral therapy, mindfulness, and cortisol habituation: a randomized controlled trial. Psychoneuroendocrinology 104, 276–285.
Manigault, A.W., Woody, A., Zoccola, P.M., Dickerson, S.S., 2018a. Trait mindfulness predicts the presence but not the magnitude of cortisol responses to acute stress. Psychoneuroendocrinology 90, 29–34.
Manigault, A.W., Figueroa, W.S., Hollenbeck, C.R., Mendlein, A.E., Woody, A., Hamilton, K.R., Scanlin, M.C., Johnson, R.C., Zoccola, P.M., 2018b. When family matters most: a test of the association between sexual minority identity disclosure context and diurnal cortisol in sexual minority young adults. Psychosom. Med. 80, 717–723.
McEwen, B.S., 1998. Protective and damaging effects of stress mediators. N. Engl. J. Med 338, 171–179.
Mereish, E.H., Poteat, V.P., 2015. A relational model of sexual minority mental and physical health: the negative effects of shame on relationships, loneliness, and health. J. Couns. Psychol. 62, 425–437.
Meyer, I.H., 2003. Prejudice, social stress, and mental health in lesbian, gay, and bisexual populations: conceptual issues and research evidence. Psychol. Bull. 129, 674–697.
Mijas, M., Koziara, K., 2020. Polska adaptacja kwestionariusza codziennych doświadczeń heteroseksizmu. Psychiatr. Pol. 54, 137–152.
Miller, G.E., Chen, E., Zhou, E.S., 2007. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychol. Bull. 133, 25–45.
Morris, M.C., Rao, U., 2014. Cortisol response to psychosocial stress during a depressive episode and remission. Stress 17, 51–58.
Ogińska-Bulik, N., Juczyński, Z., 2008. Skala pomiaru prężności–SPP-25. Now. Psychol 3, 39–56.
Parra, L.A., Benibgui, M., Helm, J.L., Hastings, P.D., 2016. Minority stress predicts depression in lesbian, gay, and bisexual emerging adults via elevated diurnal cortisol. Emerg. Adulthood 4, 365–372.
Petrowski, K., Wintermann, G.-B., Siepmann, M., 2012. Cortisol response to repeated psychosocial stress. Appl. Psychophysiol. Biofeedback 37, 103–107.
Pitman, D.L., Ottenweller, J.E., Natelson, B.H., 1990. Effect of stressor intensity on habituation and sensitization of glucocorticoid responses in rats. Behav. Neurosci. 104, 28–36.
Plöderl, M., Tremblay, P., 2015. Mental health of sexual minorities. A systematic review. Int. Rev. Psychiatry 27, 367–385.
Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28, 916–931.
Rahe, R.H., 1975. Epidemiological studies of life change and illness. Int. J. Psychiatry Med. 6, 133–146.
Rohleder, N., Gianferante, D., Fiksdal, A., Hanlin, L., Chen, X., Thoma, M.V., 2012. 125. HPA axis habituation to repeated acute psychosocial stress is related to lower sensitization of plasma interleukin-6 responses. Brain Behav., Immun. 26, S35.
Russell, S.T., Fish, J.N., 2016. Mental health in lesbian, gay, bisexual, and transgender (LGBT) youth. Annu. Rev. Clin. Psychol. 12, 465–487.
Ryan, C., Huebner, D., Diaz, R.M., Sanchez, J., 2009. Family rejection as a predictor of negative health outcomes in white and Latino lesbian, gay, and bisexual young adults. Pediatrics 123, 346–352.
Singer, S., Tkachenko, E., Hartman, R.I., Mostaghimi, A., 2020. Association between sexual orientation and lifetime prevalence of skin cancer in the United States. JAMA Dermatol. 156, 441–445.
Sobolewski, A., Strelau, J., Zawadzki, B., 1999. Kwestionariusz Zmian Życiowych (KZŻ). Przegl. Psychol. 42, 27–49.
Spittlehouse, J.K., Boden, J.M., Horwood, L.J., 2019. Sexual orientation and mental health over the life course in a birth cohort. Psychol. Med. 50, 1348–1355.
StataCorp, L.P., 2015. Stata statistical software (version release 14). College Station. TX Author.
Świder, M., Winiewski, M., Abramowicz, M., Bulska, D., Górska, P., Kryszk, K., Mazurczak, J., Rawłuszko, M., Soral, W., Strzałkowska, A., 2017. Situation of LGBTA Persons in Poland. 2015–2016 Report. Kampania Przeciw Homofobii, Warsaw.
Wüst, S., Federenko, I.S., van Rossum, E.F.C., Koper, J.W., Hellhammer, D.H., 2005. Habituation of cortisol responses to repeated psychosocial stress—further characterization and impact of genetic factors. Psychoneuroendocrinology 30, 199–211.