Adrenocortical responses of emerging adults in California in the two months following the Pulse night club massacre: Evidence for distal stress responses

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A R T I C L E   I N F O

A B S T R A C T

This study examined adrenocortical responses in the days following the Pulse nightclub massacre on June 12, 2016, among emerging adults in Northern California ($N = 202$; $M = 23.18$ years, $SD = 2.56$; 25% LGBQ-Latinx, 25% LGBQ-White, 25% Straight-Latinx, and 25% Straight-White) between June 13—August 12, 2016. As predicted, participants tested more proximally to the massacre had higher waking cortisol (intercepts) and flatter diurnal cortisol output (slopes), indicative of time-dependent adrenocortical arousal across the day. The effect of days post-massacre on waking cortisol was moderated by daily distress, with days since the Pulse massacre predicting waking cortisol significant only for participants reporting lower distress; participants who reported feeling higher daily distress had elevated waking cortisol across the testing period. These findings were independent of weekly personal stressors, and consistent across participants' demographic and identity characteristics. The violent attack at the Pulse nightclub was connected to increased waking cortisol and diurnal cortisol production for several days after the massacre, in a distal population exposed to the massacre vicariously, and especially for individuals not experiencing other, personal stressors. Heightened physiological responses to violent crimes support policy efforts to protect vulnerable communities, including violence prevention, gun control, and community-based trauma response services for those directly and indirectly affected by gun violence.

1. Introduction

Lesbian, gay, bisexual, transgender, and queer (LGBTQ) persons of color experience high incidences of violence resulting in injury or homicide. International recognition of this reality increased in the summer of 2016, when 49 people were killed and 53 were injured on Latin night at the Pulse nightclub in Orlando, Florida in the early morning hours of Sunday, June 12, 2016. Although it now is thought the killer chose Pulse at random [1], this US mass-shooting initially was identified as hate-motivated terrorism, highlighting the heterosexism-, cissexism-, and racism-motivated violence regularly faced by ethnically/racially stigmatized LGBTQ persons in the US.

Vicarious exposure to terrorism and hate crimes predicts psychological distress for days [2] and months [3] following these violent attacks, signaling threats to safety [4]. Nationwide, LGBTQ persons reported fear [5], worry for personal and LGBTQ community safety [6], and avoidance of LGBTQ bars [7] following the attack. The broader Florida population reported elevated psychological distress and acute stress disorder symptoms [8], and June 2016 marked a peak of severe psychological distress among gay and bisexual men in the 2013–2018 National Health Interview Survey (NHIS) [9]. These vicarious and distal effects of the Pulse massacre on psychological health may have had parallel physical effects. The threat to personal and community safety from an event of that magnitude may have gotten "under the skin" and disrupted stress physiology systems.

The hypothalamic-pituitary-adrenal (HPA) axis is a primary stress response system, increasing its production of cortisol following stressors and threats [10]. Bereaved spouses of victims of the 9/11 terrorist attacks showed heightened waking cortisol levels for several months after the attacks [11]. Similarly, waking and diurnal HPA axis hyperactivity is

related to victimization among stigmatized populations [12]. Whether vicarious exposure to violence like the Pulse massacre similarly elevates waking and diurnal cortisol levels is unknown, as the unpredictability of such events challenges researchers' abilities to immediately collect data in the following days and weeks [5].

1.1. Hypotheses

Coincidentally, our biopsychosocial study of ethnically/racially (Latinx and White) and sexually (lesbian, gay, bisexual, or queer; LGBQ and Straight) diverse emerging adults in Northern California launched in June 2016. We predicted that the Pulse massacre would evoke HPA axis activity in a time-dependent manner, such that waking and diurnal cortisol levels would be higher and/or flatter among participants tested more proximally to June 12. As personally-experienced distress also affects HPA activity, we examined this as a potential moderator of the effects of time on cortisol. Given the identity characteristics of most of the victims of the Pulse massacre, we also examined whether ethnicity/race, sexual orientation, and sex-designated at birth moderated the effects of time on cortisol.

2. Method

2.1. Participants

Two hundred two ($N = 202$) emerging adults ($M = 23.18$ years, $SD = 2.56$, range 18–29) participated within two months after the Pulse nightclub massacre (June 13—August 12, 2016),[1] in a fully-crossed (Ethnicity/Race X Sexual Orientation X Sex designated at birth) design study including Latinx and White emerging adults who identified as LGBQ or Straight (heterosexual) and female or male at birth. Participants' demographic characteristics are presented in Table 1. Participants were recruited through listservs, free and paid advertisements, and Pride month events in Northern California. All participants gave informed consent and received compensation.

2.2. Procedure

After in-person training, participants self-collected six saliva samples at home using absorbent oral swabs (Salivettes™, Salimetrics Inc., State College, PA), upon waking, 30–45 min post-waking, and at bedtime over two consecutive days. The post-waking sample was not included in current analyses. Participants were instructed to not brush their teeth for 2 h before, and to not consume food, drinks, tobacco, or caffeine for 1 h before, collecting each saliva sample. Programmed text messages reminded participants when to collect the samples, and participants recorded the specific time and date, and completed daily diary reports of distress, for each sample. Participants also recorded whether they had experienced unusual events, or had consumed food, drinks, tobacco, or caffeine 1 h prior to completing each sample. Saliva samples were stored in home freezers until being picked up by the first author and transferred to a −30C medical freezer. Samples were stored in the laboratory freezer at −30C until all data were collected, in order to assay cortisol in one batch.

2.3. Measures

Days since the Pulse massacre. The number of days between June 12 and the first day of saliva sampling was the primary predictor of cortisol

levels.

Daily distress. Participants rated how "stressed" and "sad" they felt at the time of each waking and evening saliva sample, from 1 = *Not at all* to 5 = *Extremely*. These eight emotion ratings were all positively inter-correlated, and therefore averaged to indicate mean daily distress across both sampling days, Cronbach's α = 0.78.

Salivary cortisol. Cortisol was assayed from the saliva samples, which were shipped to Salimetrics™ (Carlsbad, CA) and assayed in duplicate for salivary cortisol using a highly sensitive enzyme immunoassay. The minimum detection ranged from 0.007 to 1.8 μg/dL, and the intra- and inter-assay variabilities were 8.31% and 7.69%, respectively. On average, waking and bedtime salivary cortisol samples were taken at 07:46AM and 23:12PM, respectively. Of the total samples assayed ($n = 807$; only one ($n = 1$) participant did not return a bedtime sample), a few samples ($n = 9$) did not contain enough saliva for assay, and one sample ($n = 1$) was flagged by technicians for likely interference during assay and was removed from all subsequent analyses (therefore, 98.6% useable samples). The averages of the duplicate raw cortisol values of each waking and bedtime sample were computed, then log10-transformed to correct for skewness. The raw cortisol values are presented in Table 1.

2.4. Covariates

Sampling and demographic information. At each saliva sampling, participants reported on their experiences of unusual events, and consumption of food, caffeine, or tobacco in the preceding 60 min [13]. Participants' reported experiences of unusual events, and consumption of food, caffeine, or tobacco (dummy coded *No = 0, Yes = 1*) were not associated with raw or log10-transformed cortisol values (all $ts < |1.61|$, $ps > .05$). Therefore, these measures were not included in the computation of the cortisol intercepts and slopes.

Participants reported their age, sex-designated at birth (0 = *Female*, 1 = *Male*), medication usage (0 = *No*, 1 = *Yes*), height and weight, used to calculate body mass index (BMI) [14,15], and income (5-point scale; see Table 1) [16]. Participants also reported on their experiences of stressors in the past week, on the 25-item Weekly Stress Inventory [17] (e.g., *"was excluded or left out," "argued with a friend," had too many responsibilities"*). Endorsed items were rated from *1 = Happened not stressful* to *7 = Extremely stressful*, with higher mean scores indicating heightened stress within the past week; Cronbach's α = 0.89. These six covariates were included in the path analysis predicting cortisol intercept and slope from days since the Pulse massacre and daily distress. Additionally, participants' ethnicity/race (Latinx or White) and sexual orientation (LGBQ or Straight) were also included in these analyses to account for known variability in diurnal cortisol patterns specific to individuals belonging to these social group memberships [12,18].

2.5. Analytic strategy

Cortisol intercepts and slopes. Multilevel linear modeling (MLM) of the individual log10-transformed cortisol values from the two waking and two bedtime saliva samples were used to compute participants' diurnal cortisol intercepts (cortisol at waking) and diurnal slopes (change from waking to bedtime). Cortisol intercepts and slopes were derived by fitting a linear regression line predicted from that person's cortisol values, accounting for variation in collection times (centered at waking) and sampling day. Higher cortisol intercepts were indexed by more positive coefficients, and steeper slopes by more negative coefficients. To illustrate this approach, we used the following multilevel model:

Level 1 Equation

$$Y_{ti} = b_{0i} + b_{1i}X_{1ti} + b_{2i}X_{2ti} + \varepsilon_{ti} \tag{1a}$$

Level 2 Equations

$$b_{0i} = \gamma_{00} + u_{0i} \tag{1b}$$

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[1] There was brief pause in testing during July 2016 as additional funding was secured. Participants tested pre and post the data collection pause were dummy coded (*Pre = 0, Post = 1*). This dummy variable was covaried in the main path analyses to account for any potential effects of the data collection pause on diurnal cortisol intercepts and slopes.

Table 1
Descriptive statistics for this sample ($N = 202$).

	n	*%*	*M*	*SD*	*Min*	*Max*	*Skewness*
Demographic information							
Sex-designated at birth							
Female	103	51.0					
Male	99	49.0					
Gender							
Cisgender female	82	40.6					
Cisgender male	95	47.0					
Transgender female-to-male	6	3.0					
Transgender male-to-female	1	.50					
Genderqueer or non-binary	18	8.9					
Race/Ethnicity							
Latinx	100	49.5					
White	102	50.5					
Sexual orientation							
LGBQ	102	50.5					
Straight/Heterosexual	100	49.5					
Education completed							
High school	18	8.9					
Trade school or some college courses	103	51.0					
College degree	81	40.1					
Employment							
Employed	99	49.0					
Unemployed	9	4.5					
Full-time student	80	39.6					
Student and employed	14	6.9					
Income							
$0 – $19,999	116	57.4					
$20,000 – $29,999	27	13.4					
$30,000 – $39,999	25	12.4					
$40 000 – $49,999	10	5.0					
$50,000 – > $100,000	24	11.8					
Main Predictors and Outcomes							
Days since Pulse massacre			33.78	21.13	2.00	60.00	-.19
Daily distress			1.71	.58	1.00	3.88	1.21
Raw waking cortisol			.384	.246	.017	2.096	2.049
Raw bedtime cortisol			.089	.113	.002	1.052	4.299
Log_{10} cortisol intercepts			3.48E-11	0.135	−0.488	0.333	−0.412
Log_{10} cortisol slopes			3.08E-10	0.013	−0.032	0.045	0.283

$$b_{1i} = \gamma_{10} + u_{1i} \tag{1c}$$

$$b_{2i} = \gamma_{20} \tag{1d}$$

wherein Y_{ti} equaled the logarithm of cortisol for participant i at time point t (with four time points total; i.e., two per day across two days); X_{1ti} equaled the measurement occasion for participant i at time point t (i.e., 0 for waking, and 1 for bedtime); and X_{2ti} equaled the day for participant i at time point t. Following the model, b_{0i} reflected the expected log_{10}-cortisol level at waking for individual i, and b_{1i} reflected the expected change in log-cortisol from waking to bedtime for individual i. The slopes for each individual (i.e., b_{1i}) were stored and used as the dependent variable in the subsequent linear multiple regression analysis.

Prediction of diurnal cortisol. Days since the Pulse massacre (predictor) and daily distress (moderator) were each mean-centered prior to creating interaction terms. Linear multiple regression analyses with interaction terms were fitted as path models with the lavaan package Version 0.6–5 in R Version 3.6.2, adjusting for covariates. Significant interaction terms were probed with simple slope analyses at ±1 standard deviation (*SD*) of the moderator. Missing data occurred at a low frequency, with less than 1% of data missing overall. Considering the small amount of missing data, full information maximum likelihood was used to account for missing data in the subsequent analyses.

3. Results

The multiple regression linear model is presented in Table 2. Days since the Pulse massacre significantly and negatively predicted both cortisol intercepts ($b = -4.01$, 95% CI: [−6.92, −1.10]) and cortisol slopes ($b = -3.10$, 95% CI: [−6.06, −1.42]); therefore, participants tested closer to June 12 had higher waking cortisol and flatter/less variable diurnal cortisol output than participants tested later over the ensuing 2 months. The effect of days since the massacre on cortisol intercepts was moderated by daily distress ($b = 2.14$, 95% CI: [0.54, 3.73]) (see Fig. 1a). The effect of time was significant only for participants who reported lower (-1*SD*) daily distress ($b = -5.26$, 95% CI: [−8.39, −2.12]); cortisol intercepts were elevated across the testing period for participants who reported higher (+1*SD*) daily distress ($b = -2.77$, 95% CI: [−5.74, 0.201]). Daily distress did not moderate the effect of days since massacre on cortisol slopes (see Fig. 1b). Race/ethnicity, sexual orientation, and sex-designated at birth did not predict diurnal cortisol values or moderate the effect of days since the massacre on cortisol intercepts and slopes, but older participants had higher cortisol intercepts.

To further explore these effects on diurnal cortisol, a model predicting the mean log_{10}-transformed bedtime cortisol values was examined. There was a significant, negative main effect of days since the Pulse massacre on bedtime cortisol values ($b = -7.32$, 95% CI: [−14.09, −0.541]), indicative of elevated bedtime cortisol levels in participants tested closer to the date of the massacre. Daily distress was not associated with bedtime cortisol and daily distress did not moderate the effect of days since the Pulse massacre on bedtime cortisol values (please see Supplemental Table 1).

4. Discussion

Just as both LGBTQ communities [5–7,9,19] and the general population [8] experienced psychological distress following the Pulse

Table 2
Model predicting cortisol intercepts and slopes.

Outcome	Predictor	b	Estimate	SE	95% LCI	95% UCI
Cortisol intercepts						
	Days since Pulse massacre	−0.625	−4.012	1.485	**−6.922**	**−1.102**
	Daily distress	0.073	0.170	0.198	−0.217	0.558
	Days since Pulse massacre X Distress	0.190	2.137	0.814	**0.542**	**3.732**
	Weekly stressors	0.025	0.033	0.098	−0.160	0.226
	Age	0.158	0.838	0.372	**0.109**	**1.566**
	Sex-designated at birth	−0.072	−0.194	0.209	−0.603	0.215
	Race/ethnicity	0.026	0.071	0.204	−0.330	0.472
	Sexual orientation	0.018	0.050	0.192	−0.325	0.425
	Medication usage	0.026	0.078	0.244	−0.400	0.555
	BMI	−0.047	−0.946	1.419	−3.727	1.834
	Income	0.013	0.084	0.445	−0.788	0.955
	Pre/Post data collection pause	0.461	1.264	0.654	−0.018	2.545
Cortisol Slopes						
	Days since Pulse massacre	−0.502	−3.100	1.509	**−6.058**	**−0.142**
	Daily distress	0.055	0.125	0.181	−0.231	0.480
	Days since Pulse massacre X Distress	0.004	0.049	0.837	−1.592	1.690
	Weekly stressors	0.093	0.117	0.099	−0.077	0.312
	Age	−0.122	−0.620	0.364	−1.333	0.092
	Sex-designated at birth	−0.030	−0.077	0.199	−0.468	0.313
	Race/ethnicity	−0.034	−0.088	0.202	−0.485	0.309
	Sexual orientation	0.011	0.029	0.188	−0.339	0.397
	Medication usage	0.056	0.164	0.227	−0.280	0.609
	BMI	0.010	0.205	1.452	−2.641	3.050
	Income	−0.045	−0.280	0.431	−1.126	0.565
	Pre/Post data collection pause	0.467	1.232	0.638	−0.018	2.483
Covariances						
Cortisol Intercepts						
	Cortisol slopes	0.140	0.225	0.147	−0.063	0.512
Daily distress						
	Weekly hassles	0.377	0.225	0.050	**0.126**	**0.323**
Model Fit						
χ2 (3) = 3.65, *p* > .05; *RMESA* = 0.033, 90% CI [0.000, .127], *CFI* = .999, *TLI* = .969, *NFI* = .995, *SRMR* = 0.019						

Notes. b: standardized beta; *SE*: standard error; *LCI and UCI:* 95% lower and upper confidence intervals. *RMESA:* root mean square error of approximation; *CFI:* comparative fit index; *TLI* = Tucker–Lewis index, *NFI* = normed fit index; *SRMR:* standardized root mean square residual.

massacre, physiological effects of this violent and tragic event were observable in heightened waking and diurnal patterns of salivary cortisol among a diverse sample of emerging adults. Although participants in this study lived almost 3000 miles from the terrorist attack, these LGBQ and Straight, Latinx and White individuals appear to have vicariously experienced and embodied the stress of this major life event. Their HPA axis activity was elevated across the diurnal cycle, evident both at waking (intercept) and at bedtime. HPA axis hyperactivity being evident in persons tested more proximally to the date of the massacre aligns with the documented peak of severe psychological distress among stigmatized persons in June 2016 from the 2013–2018 NHIS [9]. This cumulative evidence for both psychological and adrenocortical effects in distal communities in the days and weeks following the Pulse massacre suggests that severely violent crimes may vicariously and adversely affect the acute health of a much larger portion of the population than only those who are similar to people and communities that are the direct victims of such crimes [4], although those individuals may continue to experience effects for longer (e.g., Ref. [11]).

Although we did not have an *a priori* prediction regarding how subjective daily distress would affect the association between days since the Pulse massacre and cortisol levels, it is notable that waking cortisol remained elevated across the testing period for individuals who reported feeling more daily distress even after accounting for general stressors they experienced in the week preceding testing. Personal distress is reliably associated with elevated waking and diurnal cortisol [10]. We did not ask participants to report the source of their feelings of daily distress, so it is not clear whether these feelings were attributable to prolonged psychological distress stemming from the Pulse massacre, although it could reflect consistency with a prior report of prolonged heightened adrenocortical responses in the months following vicariously witnessing the 9/11 acts of terrorism [11]. Alternatively, the

stable concordance of elevated waking cortisol levels and feeling more daily distress may have been attributable to other co-occurring societal sources of stress that all participants experienced, like the increasingly vitriolic rhetoric leading to the 2016 US presidential election cycle, or to other unknown personal sources of stress that varied across participants.

The specific identity characteristics of our participants did not moderate the effect of time on cortisol intercepts and slopes, paralleling the elevated psychological distress reported by the general Florida population following the Pulse massacre [8]. This was surprising in light of previous findings documenting that LGBTQ persons of color described the Pulse massacre as a targeted personal threat [19], and research suggesting multiple forms of oppression affect the psychological and physiological wellbeing of LGBQ Latinx emerging adults [18]. Yet, it parallels Ben-Ezra and colleagues' (2017) finding that Floridians in general, not just Latinx and/or LGBTQ Floridians, experienced elevated psychological distress following the massacre. Our specific main effect on cortisol intercepts parallels the finding of Pfeffer and colleagues (2009) that, compared to non-bereaved spouses, spouses bereaved by the 9/11 terrorist attacks had elevated waking cortisol; and our main effect of cortisol slopes extends their results by showing that vicarious exposure to violence is linked to less variable cortisol throughout the day and elevated cortisol levels persisting into the evening.

4.1. Limitations and implications

This study's limitations included lacking a subjective stress measure specific to the Pulse nightclub massacre and our reliance on waking and evening cortisol samples over the course of two days to model the diurnal slope; more samples per day and more days of sampling would have been desirable (e.g., Ref. [20]). Participants were not asked to report on duration or quality of sleep in the nights prior to the saliva
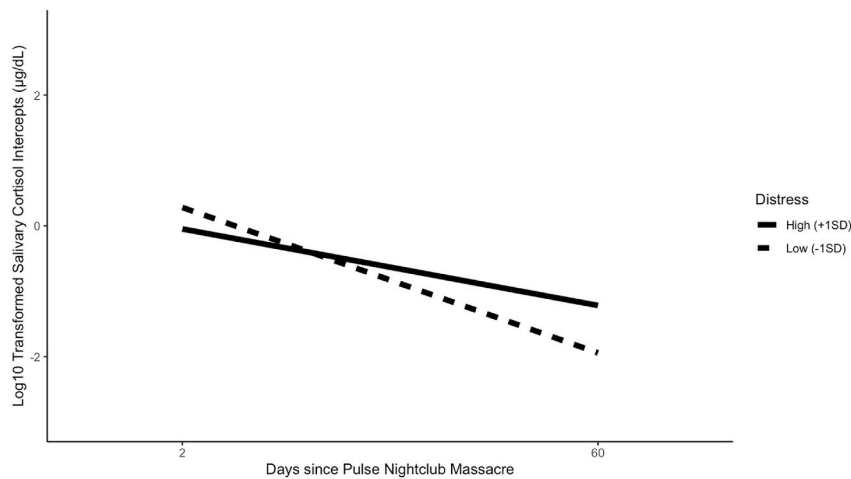
a. Subjective daily distress moderated the effect of days following the Pulse nightclub massacre on cortisol intercepts.
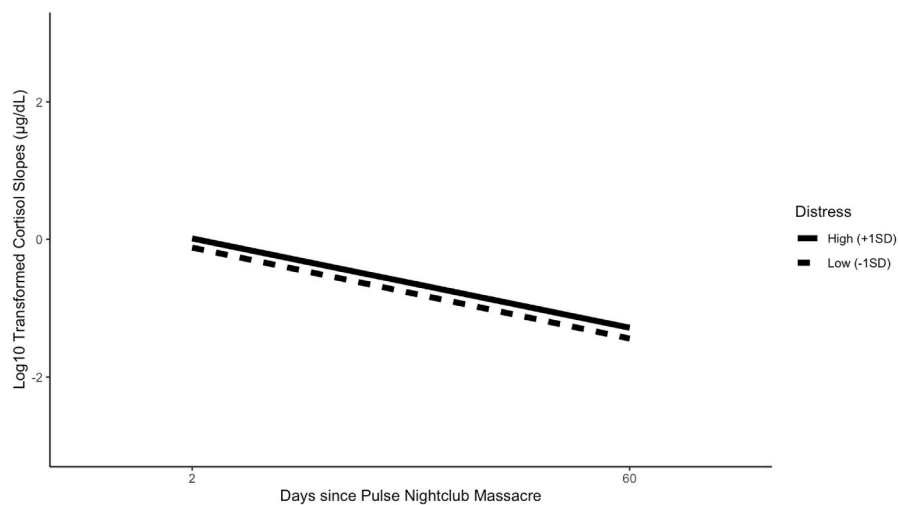
b. The main effects of days following the Pulse nightclub massacre on cortisol slopes.

Fig. 1. a. Subjective daily distress moderated the effect of days following the Pulse nightclub massacre on cortisol intercepts (waking).
Fig. 1b. The main effects of days following the Pulse nightclub massacre on cortisol slopes (diurnal).

sample collections, which are known to affect diurnal cortisol patterns [21,22]. As data collection did not proceed the Pulse massacre, this study cannot definitely demonstrate that participants' diurnal cortisol patterns were different in the days following the massacre than they were in the days preceding the massacre. Moreover, our findings were specific to a targeted convenience sample of residents in Northern California, which may not reflect the adrenocortical responses of people who suffered personal losses due to the massacre at Pulse nightclub or who vicariously witnessed the massacre elsewhere in the nation.

Nonetheless, findings from this study suggest that violent crimes targeting stigmatized groups of people may affect physiological stress responses in broader swaths of the population. These types of violent crimes victimize entire groups of people and generate personal and community threats to safety that can adversely affect perceptions of safety and psychological well-being, yet paradoxically, also can motivate personal coping responses such as changing political orientation and positions on gun control [8]. Our recent findings expand this body of work by showing that vicariously-experienced hate violence is embodied and affects physiological stress responses connected to structural and interpersonal stigma, health disparities, and gun violence.

The physiological toll of the Pulse nightclub massacre on the bodies of US residents reinforce the critical need for violence prevention, gun control, and community-based trauma response services for those affected by hate violence and gun violence.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpnec.2022.100129.

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