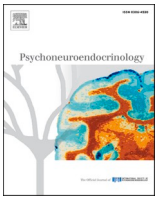
Psychoneuroendocrinology 146 (2022) 105909

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen



Trauma-induced human glucocorticoid receptor expression increases predict subsequent HPA-axis blunting in a prospective longitudinal design

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ARTICLE INFO

Keywords:
Glucocorticoid receptor
mRNA
Cortisol
HPA
PTSD
Trauma

ABSTRACT

One of the hallmarks of post-traumatic stress disorder (PTSD) is abnormalities in the HPA-axis. This includes alterations in its negative feedback regulation. Although altered glucocorticoid receptor (GR) mRNA expression is thought to play a crucial role herein, direct longitudinal evidence in humans is lacking to support this assumption. The current prospective longitudinal study assessed the consequence of repeated trauma exposure on GR mRNA expression from saliva samples in early-career police recruits (n = 112) by assessing them before and after trauma exposure. We did not observe a relationship between change in GR mRNA expression and development of PTSD symptom severity. However, the more traumatic events were experienced during police training the stronger GR mRNA expression was increased. Moreover, increases in GR mRNA expression were associated with blunted HPA-axis stress-reactivity at follow-up compared to baseline. This study provides the first longitudinal evidence of a dose-response relationship between trauma and human GR mRNA expression (extracted from saliva) changes; therefore, replication is warranted. Our finding might contribute a possible explanatory framework for blunted HPA-axis function associated with PTSD.

1. Introduction

Exposure to psychological stress can result in the development of stress-related disorders such as Post-Traumatic Stress Disorder (PTSD; American Psychiatric Association, 2013). PTSD is characterized by intrusive and recurring traumatic memories, trauma-related emotional and behavioral symptoms, and hyperarousal (American Psychiatric Association, 2013). Why some individuals are more vulnerable to develop PTSD than other individuals, remains an important question. This question is especially important to answer in order to facilitate and improve early intervention.

One of the biological alterations related to PTSD are blunted hypothalamic-pituitary-adrenocortical (HPA) axis responses. Such alterations are observed in patients compared to healthy controls or individuals that did not develop PTSD after trauma (de Kloet et al., 2006; Heim and Nemeroff, 2009; Yehuda and LeDoux, 2007). In response to a stressor, HPA-axis activation results in the secretion of cortisol in humans and corticosterone in animals, together indicated as CORT (De Kloet et al., 2005). When CORT levels are low, CORT mostly binds to high-affinity mineralocorticoid receptors (MR). However, when CORT levels rise such as after stress exposure, CORT increasingly binds to glucocorticoid receptors (GR). Binding to the GR inhibits the synthesis of CORT by inhibiting HPA-axis activation via a negative feedback loop (Gjerstad et al., 2018). It is the repeated activation of this negative feedback mechanism that may explain how chronic exposure to stress can lead to blunted CORT levels in the long run (Yehuda et al., 2006, 2005). Indeed, several meta-analyses have indicated that individuals with PTSD have lower morning CORT and 24 h CORT concentrations compared to controls (i.e., individuals exposed to trauma without developing symptoms and those that were not exposed to trauma; (Meewisse et al., 2007; Morris et al., 2012; Schumacher et al., 2019)). PTSD patients have also been shown to have reduced CORT

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https://doi.org/10.1016/j.psyneuen.2022.105909
Received 10 January 2022; Received in revised form 27 July 2022; Accepted 25 August 2022
Available online 30 August 2022

stress-responses relative to a psychiatric control group of patients with social anxiety disorder (Santa Ana et al., 2006). These findings together are suggestive of an overactive negative feedback system in PTSD.

There are a few studies that have investigated changes in the negative feedback regulation of the HPA axis in individuals with PTSD. An early investigation demonstrated a higher baseline GR expression on lymphocytes in a group of combat veterans compared to a healthy control group, regardless of whether the veterans met criteria for PTSD (Yehuda et al., 1995). Similarly, Labonté et al. (2014) demonstrated higher GR mRNA expression in a group of individuals with current and past PTSD compared to a control group that never experienced trauma. Although this suggests that trauma exposure can lead to GR mRNA expression changes, these earlier studies could not disentangle predisposing from acquired changes in GR mRNA expression after trauma exposure, something that requires prospective longitudinal studies.

Indeed, several prospective studies (van Zuiden et al., 2009, 2009; Van Zuiden et al., 2012, 2011) did find that pre-existing characteristics of several components of the GR pathway are linked to later PTSD symptom development after military deployment. For example, both (Van Zuiden et al., 2012, 2011) found that pre-existing GR number in peripheral blood mononuclear cells (PBMCs) was predictive of later PTSD symptom development. Moreover, glucocorticoid sensitivity of leukocytes (i.e. monocytes and T-cells; van Zuiden et al., 2012) and mRNA expression of (downstream) GR target genes (i.e. FKBP5 and GILZ, but not SGK1; van Zuiden et al., 2012) were found to be related to later PTSD symptom development. Also, early post-trauma GR function markers such as salivary cortisol levels (McFarlane et al., 2011) have been associated with later PTSD symptom development. However, with regards to mRNA expression of the GR, (Van Zuiden et al., 2011) found no pre-existing differences, measured from PBMCs, between individuals that would later develop PTSD and those that would not. Even though the authors investigated this in a prospective study, they did not investigate whether GR mRNA expression levels following deployment were altered. It is therefore possible that changes in GR mRNA expression are due to trauma-exposure. Another study Schür et al. (2017) investigated whether pre-deployment GR-1_F methylation was associated with post-deployment PTSD symptoms, but could not confirm such an association. However, methylation was affected after deployment which was related to the amount of trauma exposure. Together, some components of the GR pathway seem to predisposition for PTSD symptom

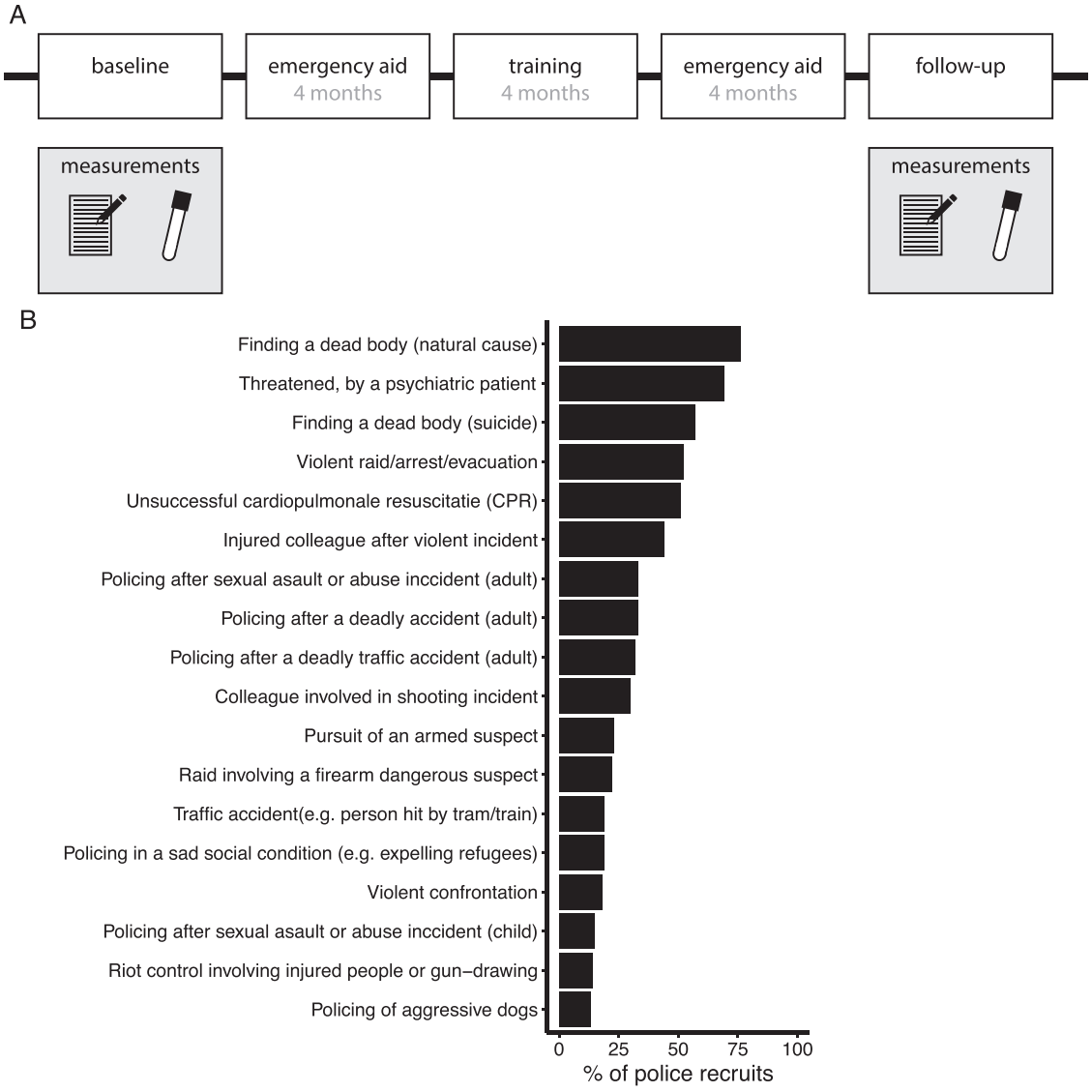

Fig. 1. A) Overview of the timeline of the study. Between baseline and follow-up, police recruits gained experience in emergency aid (two periods) as part of their training, encountering potentially trauma-related events. The measurements described here were taken during the baseline and follow-up visit. B) A list of potentially traumatic events from the Police Life Events checklist (PLES) (Carlier and Gersons, 1992) reported by the police officers between baseline and follow-up measurement. Only events are showed that was experienced at least by 10% of the police recruits.

development, whereas others are altered as a consequence of trauma exposure. To investigate whether GR-expression does change after trauma-exposure and whether such change may contribute to blunted HPA-axis regulation, there is great need for prospective longitudinal studies in relatively healthy and un-exposed individuals that are at high risk for trauma-exposure.

In this study, we assessed the impact of trauma exposure on GR mRNA expression levels and HPA-axis reactivity. We tested this impact in a relatively resilient group (n = 112) of early-career police recruits prior to their enrollment in a stressful period during their training (see Fig. 1). Throughout this training period police recruits were exposed to many potentially traumatic events. We indexed trauma symptoms using the PTSD checklist for DSM-5 (PCL-5) (Boeschoten et al., 2014; Weathers et al., 2013) and trauma exposure with the Police Life Events Scale (PLES) (Carlier and Gersons, 1992). We expected firstly, that GR mRNA expression levels would be altered during follow-up after trauma exposure compared to baseline. In line with a previous non-prospective study showing that trauma-exposure is associated with higher GR mRNA expression (Labonté et al., 2014) it is possible that GR mRNA expression levels would be increased following trauma exposure compared to before. Secondly, we expected that the change in GR mRNA expression levels would be associated with an increase in post-traumatic stress symptoms (i.e., PCL-5 scores) and trauma exposure (i.e., PLES scores) during follow-up compared to the period prior to training. Finally, following evidence of a possible altered negative feedback loop with regards to HPA-axis function (Yehuda et al., 2006, 2005), we expected that a change in GR mRNA expression levels would be associated with changes in general CORT levels (i.e., assessment during rest) and CORT reactivity (i.e. assessment during a stress induction procedure). We assessed GR mRNA expression from saliva and did not assess GR gene expression from PBMCs such as leukocytes, as was often done in previous work. The cellular mRNA expression of GR is regulated by small non-coding RNAs as well as post-transcriptional and post-translational modifications affecting the activity of GR (Oakley and Cidlowski, 2013; Schür et al., 2017). Therefore, mRNA expression of GR may reflect a part of (genetic) regulation of the HPA axis. GR gene expression does not necessarily reflect the amount of cellular GR protein, protein stability, or functional GR present in the cell. Interestingly, a recent report (Theda et al., 2018) has suggested that saliva contains a high number of leukocytes. This is not surprising given that the buccal mucosa is relatively permeable and has a rich blood supply. Therefore, evaluating GR mRNA expression from saliva may be a useful alternative, as collection of saliva is far less invasive compared to the collection of blood samples. For these reasons and for the practical advantages of doing non-invasive sampling, in the current study we have therefore chosen to measure GR mRNA expression from saliva.

2. Materials and methods

2.1. Participants

All participants were recruits from the Dutch Police Academy. 713 police recruits were assessed for eligibility, of whom 57 were excluded, 314 declined to participate, and 342 (48.0 %) provided informed consent to participate. Exclusion criteria were any current psychiatric or neurological disorder; history of, or current, neurological or endocrinological treatment; current use of psychotropic medication; and current drug or alcohol abuse. These criteria were assessed over the phone. 340 participants completed the baseline assessment, of whom 271 (79.7 %) completed follow-up, 21 (6.2 %) only completed follow-up symptom assessments, and 48 (14.1 %) were unavailable for follow-up. Study attrition was not related to demographics, baseline clinical characteristics or trauma history (see Supplementary Table 1 in Koch et al., 2021 for further details).

As we sought to test the impact of trauma exposure, we included participants who experienced their core traumatic event between baseline and follow-up (n = 222), as assessed with a clinical interview Clinician-Administered PTSD scale (CAPS-5) (Boeschoten et al., 2014). One participant was excluded from analysis because they reported having PTSD symptoms above the clinical cut-off at baseline (PCL-5 total score > 33; Weathers et al., 2013). Note we used the PCL-5 total score as our PTSD symptom measure as registered a priori in our protocol paper (Koch et al., 2017). The reason for this is that the CAPS assessment were only done at follow-up. Out of those 221, there was GR expression data available for both sessions (baseline, follow-up) for 112 participants. From those 112, there were baseline CORT levels available for both sessions for 102 out of 112 participants, and (partial) stress-related CORT levels for both sessions for 82 (or 76 for the increase) out of 112 participants. Because of missing data on these dependent variables, we could only perform the analysis on these subgroups. See Table 1 for the demographics of the current sample.

2.2. Procedure

All reported experiments were conducted as part of a large prospective study assessing the role of automatic defensive responses in the development of trauma-related psychopathology in police recruits (Netherlands Trial Registry NTR6355). See (Koch et al., 2017) for details on the general procedure and (Hashemi et al., 2021; Kaldewaij et al., 2019a,b; Koch et al., 2021; Zhang et al., 2019, 2020, 2022) for previous studies on the same sample. Baseline assessments took place when participants were still in the relatively structured environment of the police academy. Follow-up assessments took place after the first period of emergency aid experiences with potentially trauma-related events (see Fig. 1), on average after 16 months (M = 482 days, SD = 55 days, range 373–679 days). We checked whether the variation in time delay affected our outcome measures, this was not the case and therefore statistics are reported without time delay in the model. Across the session, participants completed several questionnaires and performed several behavioral and fMRI tasks (not reported here). Upon arrival in the lab (1100 for the early session and 1230 for the late session), participants provided written informed consent and were given a glass of water. This was 30 min before the saliva sample from which the mRNA extraction was done. They were instructed (in those 30 min) to not eat, drink, smoke, or chew gum. They also took a physical rest during the 30 min. To account for changes in CORT due to circadian cycle, the CORT assessment during rest was always administered between 3:00PM and 4:15PM. The start of the socially evaluated cold pressor task (SECPT; with the first measurement of timepoint −10 min) was between 4:15PM and 6:15PM when CORT levels are relatively stable (Schwabe et al., 2008). Similarly, participants were instructed to not eat, drink, smoke, or chew gum for at least 30 min before the saliva samples were taken. Each visit consisted of two fMRI sessions, and during the second session the stress-induction (i.e., via the SECPT) took place. See below for a

Table 1
Sample demographics (n = 112).

	Mean/Count	SD	Range
Age	23.2	4.7	18–43
Sex			
Male	88/112		
Female	24/112		
Oral Contraception	21/24		
Ethnicity	European (Caucasian): 107/112 African: 3/112 Other: 2/112		
Days between sessions	479	52	373–656
PCL			
Baseline	4.05	4.85	0–21
Follow-up	5.26	7.25	0–34
PLES			
Baseline	1.78	2.43	0–14
Follow-up	6.36	3.58	0–18

detailed description of each procedure.

2.3. Questionnaires

As mentioned in the protocol article of this study (Koch et al., 2017), the primary outcome measure was change in PTSD symptom severity assessed by the PTSD checklist for DSM-5 (PCL-5; Boeschoten et al., 2014; Weathers et al., 2013). The PCL-5 was used to assess PTSD symptom severity, by self-rating of all DSM-5 PTSD symptoms in the last month, ranging from 0 (absent) to 4 (extreme/incapacitating). To assess specifically the increase in PTSD symptoms, we calculated a PTSD change score (Δ-PCL) by subtracting the baseline PCL-5 score from the follow-up score.

Participants additionally filled out the Police Life Events Scale (PLES) twice to measure police work-related trauma incidence once before and during the training period (Carlier and Gersons, 1992). This is a list of 40 possible traumatic police work-related events that are violent (for example shootings or escalating riot situations) or tragic (for example finding a corpse or being confronted with severely mutilated victims). The baseline PLES inquired about all events that participants had previously encountered during their life. Some participants have experienced one or more of these events before their training and included events such as finding a dead body and being threatened by a psychiatric patient. The follow-up PLES inquired specifically about all events during the period of approximately 16 months between baseline and follow-up. The PLES score reflects the number of different encountered traumatic events. The change is trauma exposure is captured by the follow-up PLES, which is therefore referred to as Δ-PLES.

The PCL baseline score, PLES baseline score, and Δ-PLES score were log-transformed before they were included as covariates. Log-transformations were applied to correct for a skewed distribution.

2.4. Gene expression analysis

Saliva for RNA analysis was collected in the morning using Oragene RE-100 RNA collection kits (DNA Genotek Inc.) and stored for a maximum of four weeks at room temperature prior to long-term storage at minus 80 °C. To prevent batch effects, all RNA was extracted at the same time point following removal from the freezer. Total RNA from 250uL saliva was extracted using RNeasy Mini Kit (Qiagen) in combination with RNAse free DNAse set (Qiagen). RNA purity and concentration were determined using Nanodrop-2000 (ThermoScientific). Data integrity was assessed following isolation (RNA integrity number (RIN) values were > 7 following lab protocol and typically between 7 and 8). 100 ng of total RNA was reverse transcribed to cDNA using the SensiFAST™ cDNA Synthesis Kit (Bioline). RT-qPCR was performed using 2x Sensifast SYBR No-ROX-mix (Bioline) with the addition of 7,5 % DMSO with 2 ng cDNA template on a Rotor-Gene Q instrument (Qiagen). The cycling conditions comprised 2 min polymerase activation at 95 °C followed by 40 cycles at 95 °C for 5 s, 60 °C for 10 s and 72 °C for 12 s. This was followed by a melting curve analysis (67–95 °C, rising 1 °C/5 s) to ensure amplification specificity. The quantification cycle (Cq) was determined using Rotor-Gene Q Software (v2.1.0.). Data were normalized using commonly used housekeeping genes glyceraldehyde-3-phosphate dehydro-genase (GAPDH) and β-actin (ACTB). Primers were designed using Primer3 (Untergasser A 2012). We used validated primer sequences (Vandesompele et al., 2002); NR3C1: Forward 5′-AAGAGCAGTGGAAGGACAGC-3′ and Reverse 5′-GTAGTGGCCT GCTGAATTCC-3′, GAPDH Forward 5′-TGGAAGGACTCATGACCACA-3′ and Reverse 5′-TCCACCACTGACACGTTGG-3′ and ACTB Forward 5′-CTATCCCTGTACGCCTCTGG-3′ and Reverse 5′-AATGTCACGCACGA TTTCCC-3′. Average Ct values were normalized using the geometric mean of the housekeeping genes. Relative gene expression was calculated using the 2-ΔCt method.

2.5. Baseline salivary CORT assessment

Saliva for CORT level during rest was collected using Salicap (IBL, Hamburg, Germany) upon arrival both at baseline and during the follow-up visit. Salivary CORT concentrations were analysed using a commercially available chemiluminescence immunoassay with high sensitivity (IBL Inc.).

2.6. Stress induction and salivary CORT assessment

Stress responses were induced by sequential administration of a socially evaluated cold pressor task (SECPT) and a mental arithmetic task (Zhang et al., 2019, 2020, 2022). The SECPT was administered within the MRI. Participants, while lying down, were instructed to immerse their right foot in icy-cold (0–3 °C) water for 3 min. Immediately after SECPT, a 3-minute mental arithmetic task was administered. Participants were instructed to count back out loud from 2053 in steps of 17 as quickly and accurately as possible. During the stress induction, two experimenters were present. The primary experimenter in charge of the stress induction was new to the participants (e.g., had no contact with the participants throughout the day) and wore a white lab coat. The assistant experimenter was familiar to the participant as he/she was with the participant for the whole afternoon. The gender of at least one of the experimenters was opposite to the gender of the participants. The full stress-induction procedure lasted approximately 8 min, including instructions. 3 ml of saliva was collected into Salivette™ collection tubes (Sarstedt, Germany), which were collected at five Sampling points, relative to the start of the SECPT, at 10 min intervals (i.e., −10 min, 0 min, +10 min, +20 min, +30 min). Note that salivary CORT levels were not always available for all participants and all sampling time points. This was either due to a technical error during the baseline session, for 21 (out of 112) participants time point 5 was missing or this was due to immeasurable salivary CORT levels. As explained above, 82 participants had (partial) stress-related CORT levels for both sessions (for those ~5 % of the samples were missing) and 76 had complete data on sampling time point 0 min and + 20 min (used to calculate the baseline-to-peak measure; Zhang et al., 2019, 2020, 2022). Salivary CORT concentrations were analysed using a commercially available chemiluminescence immunoassay with high sensitivity (IBL Inc.). CORT levels were first assessed across all five Sampling points (i.e., this measure corresponds to the Area Under the Curve with respect to ground; Pruessner et al., 2003). In addition, cortisol level increases were defined by the difference between time 20 min and baseline 0 min (i.e. to obtain a baseline-to-peak measure) in line with a previous study (Zhang et al., 2019, 2020, 2022).

2.7. Data analysis

All statistical analysis were performed using R (R Core Team, 2019). Linear mixed-effects model were performed using the *afex* package and *mixed* function (Singmann et al., 2019). Correlations were performed using the *Hmisc* package and the *rcorr* function (Harrell, 2019) for correlations across participants. Alpha was set at 0.05 throughout. Standardized B's and SE's are reported.

We first assessed (Section 3.1) whether trauma exposure and PTSD symptom development changed between baseline and follow-up in the current sample (n = 112) using two models with PCL-5 and PLES scores as the dependent variable. The two models contained Session (Baseline, Follow-up), as a fixed effect, and Subjects as a random effect.

For our main analyses, we conducted four separate models with different dependent variables namely 1) mRNA GR expression (n = 112; Sections 3.2), 2) CORT during rest (n = 102; Sections 3.3), 3a) CORT during the SE-CPT (n = 82; Sections 3.4), 3b) baseline-to-peak CORT during the SE-CPT (n = 76; Section 3.4). As explained in Section 2.1 Participants each of these models included a different sample size due to missing data of the dependent variable. We maximized the sample sizes

by including participants with partial missing data. All models included the factors Session (Baseline, Follow-up), ΔPCL-5 (follow-up minus baseline) score, log-transformed PCL baseline score, log-transformed PLES baseline score and log-transformed Δ PLES score as fixed effects, and Subjects as a random effect. For the Stress-related salivary CORT model, the factor Sampling (5 time points) was added in addition as a fixed effect. Additionally, all models included Age, Sex, Childhood Trauma Scores (CTQ), the time of day participants were tested (Early, Late) for each session as covariates of no interest.

Subsequent follow-up tests or checks that were conducted will be clearly explained in the results section.

3. Results

3.1. Trauma exposure and PTSD symptom development

We first tested whether, in line with previous reports on a larger sample (Kaldewaij et al., 2021; Koch et al., 2021), participants within our subsample of 112 experienced a greater number of traumatic events and symptom severity after exposure (i.e., follow-up) compared to before (i.e., baseline). Indeed, participants experienced more traumatic events between baseline and follow-up than before the baseline measurement ([Estimate=-0.6, SE=0.039, F(1,111)=234.281, p=4.05e-29]; Fig. 2). Also partly in line with findings from the full sample of this study (Kaldewaij et al., 2021), there was a numerical rise in PTSD symptomatology across participants, although the increase in PTSD symptom severity from baseline to follow-up was no longer statistically significant within our subsample [Estimate=-0.097, SE=0.059, F(1,111)=2.733, p=0.101]. Importantly, however, the number of traumatic events experienced between baseline and follow-up did significantly correlate with trauma symptom increase [r(110)=0.27, p=0.004] indicating that participants that experienced a greater number of traumatic events experienced stronger symptom severity.

3.2. Exposure to trauma is associated with an increase in GR mRNA expression

Next, we investigated a potential change in GR mRNA expression after trauma exposure. We did not observe a significant increase in GR mRNA expression levels from baseline to follow-up [Estimate=-0.069, SE=0.073, F(1,102)=0.898, p=0.345]. Moreover, we did not observe a significant relationship between the change in GR mRNA expression levels and change in PTSD symptoms [Estimate=0.101, SE=0.068, F(1,102)=2.226, p=0.139]. However, the change in GR mRNA expression levels from baseline to follow-up was associated with the number of traumatic events experienced in that interval [Estimate=-0.145, SE=0.073, F(1,102)=3.946, p<0.05]. Those participants that experienced more traumatic events showed a stronger increase in GR mRNA expression levels from baseline to follow-up. This increase in GR mRNA expression was not associated with the number of traumatic events experience before baseline assessment [Estimate=0.097, SE=0.069, F(1,102)=1.989, p=0.161]. Moreover, the association was not dependent on including CTQ as a covariate, as the relationship between the change in GR mRNA expression levels and the number of traumatic events experienced during the training period remained significant after excluding CTQ from the model [Estimate=-0.146, SE=0.073, F(1,103)=3.982, p<0.05]. Thus, GR mRNA expression levels increased after trauma exposure as a function of the amount of trauma exposure during the training period. See Fig. 2.

The prospective longitudinal nature of our design enabled us to additionally verify whether there were already baseline differences in GR mRNA expression, predisposing for symptom development, for instance as a result of pre-baseline trauma-exposure. However, an exploratory analysis revealed there was no significant relationship between baseline individual differences in GR mRNA expression and change in trauma symptom development (baseline to follow-up PCL-5 scores) [Estimate=-0.028, SE=0.059, F(1,110)=0.23, p=0.633]. There was also no significant correlation between baseline levels of GR mRNA expression and the number of traumatic events experience before baseline [[r(110)=.07, p=.46] which may not be surprising given the relatively healthy sample of young recruits with relatively low pre-baseline trauma exposure-levels.

3.3. Change in GR mRNA expression is not associated with change in CORT levels during rest

Next, to investigate the potential consequences of changes in GR mRNA expression levels on HPA-axis functioning, we tested whether there was an association between GR mRNA expression levels and CORT levels during rest.

First, salivary CORT levels during rest were overall significantly lower during the follow-up compared to baseline assessment [Estimate=0.295, SE=0.075, F(1,91)=15.476, p=1.63e-04]. However, individual's change in GR mRNA expression levels was not significantly related to their change in CORT levels [Estimate=-0.086, SE=0.066, F(1,91)=1.712, p=0.194]. Thus, we did not observe any relationship between change in GR mRNA expression levels and change in CORT levels measured during rest.

3.4. Change in GR mRNA expression is associated with change in stress-related CORT levels

Finally, we tested whether the change in GR mRNA expression levels was associated with altered HPA-axis reactivity to a stressor. Stress-

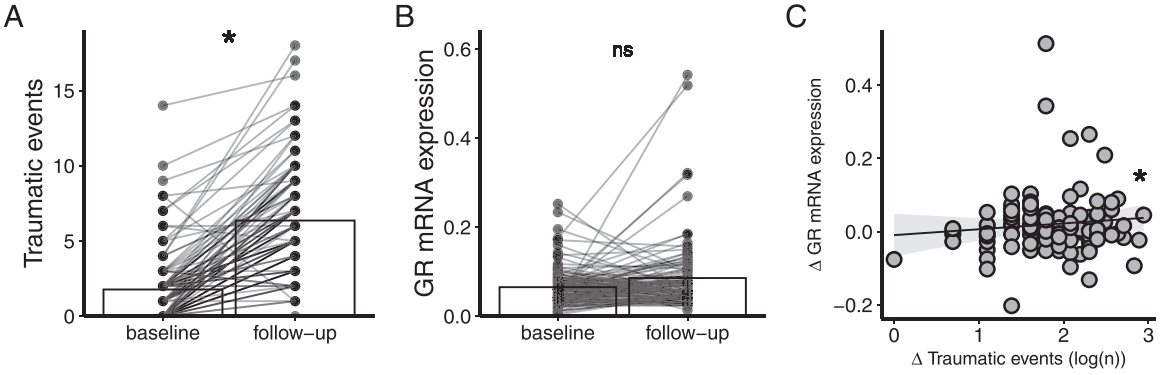

Fig. 2. A) The number of police-related traumatic events that occurred before baseline-assessment (baseline) and between baseline and follow-up (note that 'follow-up' only contains events between baseline and follow-up). B) Baseline and follow-up GR mRNA expression levels. C) Positive relationship [Estimate=-0.145, SE=0.073, F(1,102)=3.946, p<0.05] between the increase in GR mRNA expression levels and the number of traumatic events that occurred between baseline and follow-up.

induction was successful as indicated by salivary CORT level increase over Sampling time (5 time points) for both baseline (i.e. before trauma exposure) and follow-up (i.e. after trauma exposure) [Estimate=-0.143, SE=0.074, F(4,594.9)=6.508, p=3.95e-05] and a significant increase from Sampling time point 0 min to + 20 min, our baseline-to-peak measure, [Estimate=-0.352, SE=0.128, F(1,65)=7.605, p=0.008] specifically. Even though the magnitude of this acute stress-induced increase [+ 20 min minus 0 min] did not significantly differ between baseline and follow-up assessments [Estimate=-0.1, SE=0.097, F(1,65)=1.066, p=0.306; (nor was there a Sampling time (5 time points) by Session (Baseline, Follow-up) interaction [Estimate=-0.077, SE=0.077, F(4,594.72)=0.546, p=0.702])], the absolute salivary CORT levels across all 5 Sampling timepoints were higher during follow-up compared to baseline [Estimate=0.025, SE=0.075, F(1,597.62)=6.383, p=0.012], see Fig. 3A.

Critically, the change in GR mRNA expression levels from baseline to follow-up was significantly associated with the difference in absolute stress-related CORT (across all 5 Sampling time points) from baseline to follow-up [Estimate=0.026, SE=0.078, F(1,522)=5.981, p=0.015]. Namely, those participants with a stronger increase in GR mRNA expression levels (from baseline to follow-up) showed less stress-related CORT levels (across all 5 Sampling time points) during follow-up compared to baseline Fig. 3B and C. To check whether this association was not driven by potential outliers on the Δ GR mRNA expression levels (see Fig. 3C), we executed the model again but now we ranked the Δ GR mRNA expression variable. The interaction remained significant [Estimate=0.022, SE=0.074, F(1,597.77)=15.077, p=1.15e-04]. Therefore, the association between the change in GR mRNA expression levels from baseline to follow-up and the difference in absolute stress-related CORT (across all 5 Sampling time points) from baseline to follow-up was not likely driven by outlier data points. In other words, CORT stress-responsiveness, during a window stretching from stress-anticipation till after stress induction, was relatively blunted after a period of high trauma-exposure.

The change in GR mRNA expression levels was not significantly associated with the difference in an acute stress-induced increase [baseline-to-peak measure: + 20 min minus 0 min] during follow-up compared to baseline [Estimate=0.026, SE=0.071, F(1,65)=0.139, p=0.71]. Moreover, further testing revealed that changes in GR mRNA expression levels were not significantly associated with absolute stress-related CORT levels (across all 5 Sampling time points) during baseline [Estimate=0.105, SE=0.114, F(1,70.85)=0.856, p=0.358], nor follow-up [Estimate=-0.052, SE=0.098, F(1,71.33)=0.288, p=0.593], indicating that change in GR mRNA expression levels was specifically associated with the change in absolute stress-related CORT levels.

4. Discussion

In the present study, we investigated the consequence of repeated trauma exposure on GR mRNA expression levels from saliva in a relatively resilient group of early-career police recruits before and after they were enrolled in a stressful period part of their training. We found that GR mRNA expression levels were altered during a follow-up measure (i.e., after trauma exposure) compared to a baseline measure (i.e., before trauma exposure) depending on the number of traumatic events the police recruit experienced during training. The more different types of traumatic events experienced by a police recruit, the stronger the GR mRNA expression levels were during follow-up compared to baseline. The change in GR mRNA expression levels was not significantly related to PTSD symptom development. Critically, the increase in GR mRNA expression levels was associated with a reduced HPA-axis reactivity in response to a stressor. Together, these results support the view that trauma exposure can result in increased negative feedback regulation of the HPA-axis.

The number of traumatic events a police recruit experienced, was associated with an increase in GR mRNA expression levels during police training. This finding falls in line with aforementioned studies showing larger number of baseline GR on lymphocytes in a group of combat veterans (Yehuda et al., 1995) and higher GR mRNA expression in individuals with current and past PTSD (Labonté et al., 2014). One previous study reported no pre-existing differences in GR mRNA expression levels between individuals that would later develop PTSD after trauma exposure and those that would not, but did not investigate whether trauma exposure affected GR mRNA expression (Van Zuiden et al., 2011). We extend these previous findings by showing that individual differences in the amount of trauma exposure, which we were able to

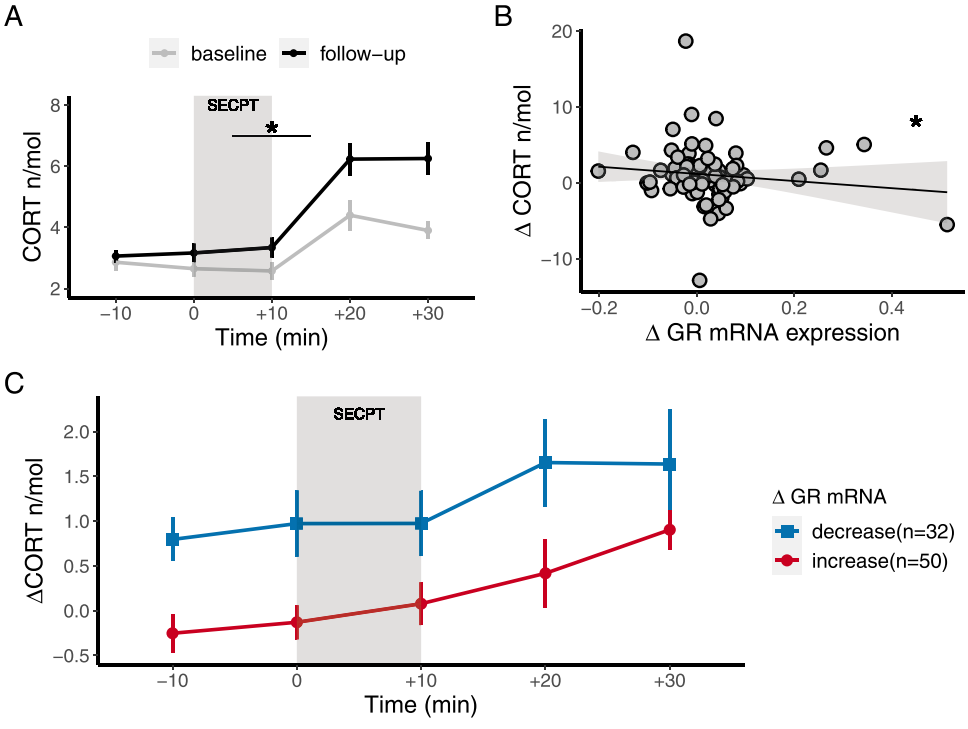

Fig. 3. (A) Stress-related CORT levels are elevated during follow-up compared to baseline. (B) The greater the increase in GR mRNA expression is from baseline to follow-up, the smaller the degree of increase in stress-related CORT is during follow-up compared to baseline [Estimate=0.025, SE=0.075, F(1,597.62)=6.383, p=0.012]. (C) For visualization purposes only and to accommodate the correlation plot shown in B, we split up the sample into 2 groups. The group with mainly a decrease in Δ GR mRNA expression levels shows the highest increase (baseline to follow-up) in stress-related CORT levels. While the group with an increase Δ GR mRNA expression levels shows the lowest increase (baseline to follow-up) in stress-related CORT levels. SECPT=socially evaluated cold-pressure test.

quantify using the Police Event Scale (PLES), predicted a later increase in GR mRNA expression. Importantly, our study differs from previous studies on the HPA-axis in trauma exposed individuals (Labonté et al., 2014; Van Zuiden et al., 2011; Yehuda et al., 1995) such that our sample consisted of a relatively resilient group of early-career police recruits. Our data thus show that there is a dose-response relationship between the number of traumatic events participants experienced and the increase in salivary GR mRNA expression levels.

Critically, we found that the change in GR mRNA expression levels was subsequently related to a reduction in HPA-axis reactivity during the anticipation and response to a stressor. Namely, the larger the increase in GR mRNA expression levels during follow-up compared to baseline, the lower the stress-related CORT levels. In line with this finding, one study showed that PTSD patients displayed reduced CORT stress-responses relative to a control group of patients with social anxiety disorder (Santa Ana et al., 2006). Another study found that healthy adults with a history of childhood adversity had lower stress-induced CORT levels in response to an acute stressor compared to a control group with lower levels of childhood adversity (Schwaiger et al., 2016). Importantly, we show that a relatively "blunted" (during follow-up compared to baseline) CORT stress-response is associated with the trauma-related increase in mRNA. As our finding is correlational, we speculate that the increase in GR mRNA expression levels consequently, as part of the negative feedback loop of the HPA-axis, may causally lead to a reduction in HPA-axis reactivity. Alternatively, however, it is also possible that the association we found is due to a causal relationship the other way around. Namely, reduced stress-related HPA reactivity could result in an increase in GR mRNA expression.

Therefore, it would be important for future studies to test the activated HPA-axis throughout a period of trauma exposure to better understand the negative feedback loop as a whole. For example, to examine whether the HPA-axis is affected at the level of the release of corticotropin-releasing hormone (CRH) from the hypothalamus or the synthesis and secretion of adrenocorticotropic hormone (ATCH) from the pituitary. This would require continuous blood sampling, which was not feasible in the current study design. In this study we have obtained GR mRNA expression levels from saliva samples instead of a more often used method using blood samples (e.g., Van Zuiden et al., 2011). The collection of saliva is non-invasive compared to the collection of blood samples. It is therefore a suitable method (Ostheim et al., 2020) to use in a large longitudinal study in which blood sample is not possible but saliva sampling is. Additionally, to build upon the findings of the current study, it would be interesting for future studies to explore to what extent GR mRNA expression level increases are related to the amount of GR protein and the binding capacity of the GR receptor. Particularly as mRNA expression does not necessarily reflect the amount of GR protein expression, as regulatory events impacting both the translation and post-translational modification can occur (De Sousa Abreu et al., 2009). It is unknown to what extent GR mRNA expression levels from blood and saliva are representative of the amount of functional GR in the brain and rest of the body. The impact of specific GR isoforms in saliva is unknown. Therefore, it may prove useful in the future to assess GR isoforms 1B given its increased expression in lymphocytes in those with life-time PTSD (Labonté et al., 2014). Future investigations exploring the GR mRNA expression in relevant post mortem human brain tissue and biological fluids in traumatized individuals could shed light on this question.

Apart from these considerations, a couple of other strengths and limitations are relevant to consider for the interpretation of our findings. This is the first prospective longitudinal study on the effects of trauma on GR mRNA expression changes using saliva samples. A strength is the selection of a relatively healthy sample at baseline that is at high risk for trauma exposure. This enables disentangling predisposing from acquired changes in GR mRNA expression. However, due to the exploratory nature of the cortisol associations, effects were reported without applying a multiple comparison correction. Although analyses tested distinct hypotheses, these findings need to be replicated. Also, further research is needed to investigate whether the relation between trauma exposure and changes in GR mRNA expression may be (partly) mediated by other factors such as the amount of physical activity or social support experienced. In addition, the second assessment of the SE-CPT was a repeated measurement whereas the baseline assessment was the first experience with this procedure. Future investigations should therefore include a longitudinal control group of participants that did not experience trauma.

In conclusion, we found that the number of different traumatic events police recruits experienced during their training was associated with an increase in GR mRNA expression levels. Moreover, the increase in GR mRNA expression was related with a blunted stress-related CORT response after their training compared to before trauma exposure. Together, we found evidence that trauma exposure may lead to an increased negative feedback mechanism of the HPA-axis as measured with an increase in GR mRNA expression and subsequent reduced HPA-axis reactivity. Our results suggest a dose-response relationship between experienced trauma and HPA-axis functioning (i.e., GR mRNA expression) in a relatively healthy group of individuals.

Funding

This study was funded by the Netherlands Organization for Scientific Research (NWO VICI-grant 453–12–0010) and a consolidator grant from the European Research Council (CoG2017 772337), awarded to KR. In addition, this project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 603016 (MATRICS) awarded to JCG. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

CRediT authorship contribution statement

RK, WZ, MMH, SK, FK, JG, and KR designed the study. RK, WZ, and MMH carried out the data collection. RAK and JG performed the mRNA extraction and were responsible for the gene expression studies. RAK, RK, LDV verified the underlying data. LDV carried out the statistical analysis and produced figures. LDV and RAK wrote the first draft of the manuscript and all authors contributed to editing and commenting on the final version.

Competing interest

All authors declared no competing interests.

Data availability

The data reported is available from the corresponding authors upon request.

Acknowledgments

We thank all participants for their willingness to participate in this study. We thank A. Smit and other personnel of the Dutch Police Academy for their valuable help with recruiting participants and facilitating this study. The authors gratefully acknowledge contributions of I. Kersten, T. Döpp, N. de Valk, L. Bovy, and L. Nuijen, G. Bertou, I. Hulzink, J. de Brouwer, K. van Groesen, N. Flipsen, M. Hartgerink, B. Becker, M. Zoet, D. van Benthem, P. van Houten in participant recruitment and data acquisition and V. van Ast for her help with setting up the study.

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