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Amygdala activity during human freezing prospectively predicts trajectories of PTSD development in Dutch police officers

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ABSTRACT

Objective: People working in risk professions are repeatedly exposed to potential traumatic events (PTEs) making them more vulnerable for development of psychopathology such as posttraumatic stress disorder (PTSD). Even though the majority seem to be resilient to stressor exposure, a small group seem to be at higher risk for development of stress symptoms. Looking at symptom development over a longer time period enables to identify different trajectories. Investigating which neural- and physiological baseline measurements predict this higher vulnerability allows for applying early interventions to improve resilience aiming to prevent development of full-blown PTSD.

Methods: Within a cohort of 140 Dutch police recruits, four measurements were collected over a period of six years including a baseline measurement before trauma exposure. Stress symptom trajectories were identified using a nonparametric k-means cluster analysis for longitudinal data (KmL). Baseline dentate gyrus (DG) volume, anterior prefrontal cortex (aPFC) activity, amygdala activity during freezing and local salience network (SN) connectivity changes were included as neural predictors. Somatic predictors included hair cortisol, salivary cortisol and heart rate variability (HRV) measurements at baseline. Multiple logistic regression models were used to investigate the predictive effect of baseline neural- and physiological measurements on the cluster distribution.

Results: Two distinct stress symptom trajectories were identified. First a resilient group showing relatively low symptom development over time and secondly an increased symptom group with significant higher symptom scores over all timepoints and a temporal increase right after trauma exposure. Baseline amygdala activity during freezing was able to predict the cluster distribution (β = 7.455; p = 0.025) above and beyond other somatic predictors.

Conclusions: Increased amygdala activity during defensive responses before trauma exposure may serve as a predisposing vulnerability factor for trauma-related trajectories. Early interventions focussing on reducing this amygdala activity may improve resilience in police officers preventing development of PTSD symptoms.

1 INTRODUCTION

Individuals with a risk profession are more likely to develop stress symptoms, such as police officers. They are exposed to different nonviolent work-related stressors such as suicide (attempts), accidents, cardiopulmonary resuscitation (CPR) or deceased persons. Exposure to violent stressful events also occur regularly involving the threat or danger of harm to themselves, their peers and citizens in society they represent. Routine work stressors including long- and irregular shifts, discrimination and insufficient resources come on top of these potential traumatic events (PTEs) (2). Development of psychopathology such as posttraumatic stress disorder (PTSD) after PTEs is well established (3). It is known that the majority of individuals exposed to trauma are resilient to stressors (4). Symptoms of PTSD involve avoidance, intrusion and reminders of the traumatic event together with nightmares, flashbacks, mood and arousal problems (5, 6). Although training and selection procedures are focused on resilience, still 35% of the police officers develop PTSD within a year after first trauma exposure (7). Police officers therefore seem to be susceptible for psychological distress and these stressors are strong predictors for development of psychopathological disorders such as PTSD (2, 8). Even though these numbers show a real impact on risk professions, it remains unclear which mechanisms are a predisposing factor for resilience and trauma susceptibility. Gaining more insights in these mechanisms is important for the development of prevention methods or for the use of early intervention methods as soon as first symptoms appear.

Predisposing factors for trauma susceptibility are often not taken into account during diagnosis of PTSD. Qualifying stress symptoms as PTSD symptoms based on the DSM-V requires a link of the symptoms to the trauma exposure both temporally and conceptually (9). Another criterium is that symptoms arose or worsened after trauma exposure together with the presence of symptoms for more than a month that are resulting in clinically significant distress or functional impairment (9). Individuals with the same diagnosis can differ substantially in the set of symptoms they experience (10). Responding to stressors is an complex interplay between environmental and biological systems that involve multiple overlapping mediators ranging from genes to neurocircuits (11, 12). Stress can alter temporary or even permanently sleep (13), memory (14), cognition (15), arousal (16), mood (17) and approach avoidance behaviors (18). Investigating these temporal alterations over a longer time period could be insightful, especially when extending the research looking into the effect of (neurobiological) mechanisms on stress symptom development. Shifting from a cut-off approach used in diagnostic tools towards a more dimensional approach could gain more insights in better understanding of stress symptom development, improvement of treatments and ultimately designing prevention methods (10, 12).

Diagnosis of PTSD is often based on a snapshot of symptoms without taking into account symptom changes over time and the heterogeneity of the disorder. Trajectory approaches could therefore be very promising looking at longitudinal symptom development. Studies in the US have examined different longitudinal outcome patterns after trauma exposure (Figure 1). First, the majority of police officers tend

to adapt well to regular PTE exposure, called *resilience* (1). Secondly, only a small group of individuals develop significant PTSD symptoms right after PTE exposure and retain high symptom scores over time (*chronic*). A third group report the same increase in symptoms after PTE exposure, but the symptoms (slowly) decrease after a certain time period (*recovery*). A fourth group can be distinguished including police officers showing a delay in symptom development (*delayed-onset*). It is unknown if these same patterns of stress symptom development can also be identified in Dutch police officers and what the underlying mechanisms are for these classifications. Neurobiological mechanisms could be important predictors for these trajectories which may be useful for the implementation of early interventions, prevention methods or changes in selection procedures. Focus of this project will be to identify patterns of stress development in Dutch police recruits that are exposed to trauma. Next to finding these trajectories, possible neural- and physiological baseline measurements will be used to investigate potential predictors for these trajectories.

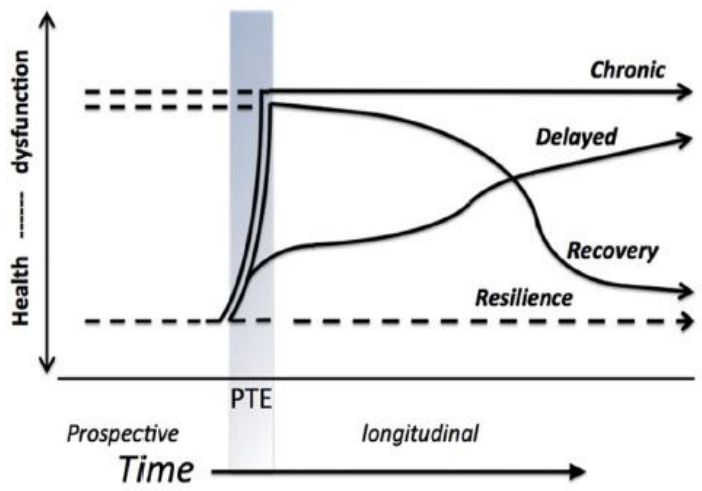

Figure 1 – Four different possible trajectories over time after potentially traumatic events (PTE): resilience- and recovery trajectories together with chronic- and delayed disruption in functioning (1).

Acute stress activates the autonomic nervous system and the hypothalamus-pituitary-adrenal (HPA) axis. Both systems are implicated in the pathophysiology of PTSD (19). Within the automatic nervous system, the dynamic interplay between the sympathetic- (SNS) and parasympathetic nervous system (PNS) causes attenuations in heart rate variability (HRV) after stress exposure. Based on the model designed by Thayer and Lane individual differences of HRV at rest is an indicator for flexibility in emotional-, cognitive and behavioural control (20-22). Where the SNS responds to stressors via the release of catecholamines, the PNS is the brake of the system reducing cardiovascular activity via vagal innervation until baseline levels are reached. Suppressed activity of PNS consequently results in reduced HRV increasing vulnerability of the

development of psychopathological disorders such as PTSD (19, 23). The hypothalamic-pituitary-adrenal (HPA) axis induces release of cortisol from the adrenal gland in blood, urine and saliva (24). Cortisol production also occurs in the skin within hair follicles via the peripheral HPA axis (25). Both hair- and salivary cortisol suppression occurs when stress symptoms maintain over a longer time period (26, 27) and seem to be markers for PTSD symptomatology (27, 28).

Neural aspects also play an important role during (acute) stress, including the prefrontal cortex (PFC), amygdala, hippocampus and resting-state network connectivity changes (29, 30). In response to acute stressful events, learning and memory is enhanced by hippocampal communication, however hippocampal activation is extremely sensitive to different emotional levels. Amygdala activity is involved in hippocampal memory storage by mediating glucocorticoid effects (31) and plays an important role during threat by activating defensive reactions and fear learning (32, 33). PFC functioning is involved in emotion regulation and the modulation of autonomic and neuroendocrine stress responses (34, 35). The PFC together with the hippocampus also play an important role in the negative feedback loop of the HPA-axis (36). Brain regions are organized into functional networks that interact with each other. Three functional resting state networks (RSNs) are involved during acute stress called the central executive network (CEN), the default mode network (DMN) and the salience network (SN) (30, 37, 38). Because it is known that these neural substrates are involved in stress responses, they could be promising predictors for the development of PTSD symptoms.

Data used in this project is part of a prospective longitudinal study investigating stress symptom development after trauma exposure. Earlier work done in this project showed that pre-trauma smaller volume of the hippocampal subregion DG predicts higher vulnerability of stress symptom development (39), as well as weak emotion regulation capabilities reflected by aPFC activity (40). Increased dorsal amygdala activation during anticipation of threat where freezing occurs predicted symptom development after trauma exposure (41). During acute stress, connectivity changes occur in the salience network (SN) important to detect and coordinate neural resources in response to behaviourally related stimuli (30, 38). Weakened connectivity between SN core regions is predictive for vulnerability of PTSD symptom development (42). These studies focused on change in PTSD symptoms right after trauma exposure. Our project builds further on these results investigating how these predictors influence longitudinal symptom development. The first hypothesis is to find at least two different patterns of stress symptom development where the majority of the recruits will be resilient to stress exposure and another small group showing increased PTSD symptoms over time. Besides, patterns showing a higher vulnerability of PTSD development are hypothesized to be associated with physiological predictors including lower HRV and suppressed cortisol levels for both salivary cortisol and HCC. Smaller DG volume, less aPFC activity, increased dorsal amygdala activation and decreased SN connectivity changes are hypothesized to be the neural predictive factors for the different trajectories reflecting increased vulnerability of PTSD symptom development over time.

2 MATERIAL AND METHODS

2.1 Participants and procedures

Participants were recruited from a prospective study designed to assess developmental changes in trauma-related psychopathology in Dutch Police recruits. Details on the general procedure and exclusion criteria of this prospective study can be found in the paper of Koch et al. (2017) (43). To investigate predictors of individual differences in post-traumatic symptom development, this study did a baseline measurement before trauma exposure (N=346) and a second measurement after trauma exposure (N=277). Time between measurements was on average 16 months (*M* = 483 days, *SD* = 57 days, range 349-679 days). Measurements were done in Nijmegen at the Donders Centre for Cognitive Neuroimaging and consisted of multiple experiments collecting psychological, psychophysiological, functional MRI (fMRI) and structural MRI (sMRI). Procedures were similar for both baseline- as after-trauma measurements. A follow-up study was designed to investigate these relationships further over a timeframe of three years with digital questionnaires. In total, six measurement rounds of questionnaires will be sent out every six months after participants gave digital informed consent. At the time of data analysis, the 3th follow-up measurement round was still in progress. Questionnaires were collected using Castor EDC (44). All participants from the prospective study were recruited again for the follow up study (N = 346), of whom 61 (17.6%) declined to participate, 141 (40.8%) participants gave digital informed consent, 4 participants were excluded due to missing informed consent (1.2%). The remaining 140 recruits (40.5%) were unavailable for follow-up. One participant scored at baseline measurement above the clinical cut-off PCL-score, and therefore excluded from analysis. To study and predict development of trauma-related symptoms, participants that reported trauma experience were included in the analysis (N = 140, 107 males, M_{age} = 29.5, SD_{age} = 5.52, range = 23 – 50 years).

2.2 Trauma-related questionnaires

Both PCL-5 and PLES questionnaires were conducted at all four timepoints. Additional control measures were taken into account and described in detail in section 6.2 Additional questionnaires.

2.2.1 PTSD CHECKLIST FOR DSM-5 (PCL-5)

PCL-5 is a measurement instrument to assess stress symptom severity consisting of 20 self-reported items mapping all DSM-5 PTSD symptoms from the last month. Items are measured on a 5-point Likert-scale ranging from 0 (“Not at all”) to 4 (“Extremely”). PCL-5 scores were calculated by the sum of all 20 items. A score of >33 is the clinical cut off for baseline measurements (45). The PCL-5 had good internal consistency, convergent and discriminant validity (Cronbach’s alpha = 0.747).

2.2.2 POLICE LIFE EVENTS SCALE (PLES)

Questionnaire assessing the number of work-related stressful events and the impact of these events on their life (46). PLES consisted of 42 yes (1) / no (0) answers. Follow-up question was generated when participants filled in yes about the influence in their lives based on a 5-point Likert-scale ranging from 0 (none) to 4 (very much). The number of traumatic events (PLES) had a good internal consistency (Cronbach's alpha = 0.755).

2.3 Predictors

All reported predictor measurements were collected during the baseline measurement of the prospective longitudinal study described above (43). Data that did not meet the normality criteria were first log-transformed. All variables were standardized to reliably compare the predictors in the model.

2.3.1 HAIR CORTISOL

Hair cortisol concentration (HCC) sampling was done according to the same procedures described in Hashemi et al. (2019). Only hair strands with a length of 1-3 cm were collected. Sampling was restricted to participants with sufficient length of scalp hair. HCC was established using a LC-MS/MS-based method (47). In total, a sample of N = 111 was included for analysis.

2.3.2 ACUTE STRESS SALIVARY CORTISOL

Acute stress was induced combining the socially evoked cold pressure task (SECPT) and the mental arithmetic (MA) task. Both psychophysiological and subjective stress responses are evoked with these tasks (48, 49). Same procedure was used in previous studies (18, 38) where the participants were asked to immerse their right foot in ice-cold water (0-3°C) for a duration of three minutes. Right after the SECPT, participants started the MA task that involves counting back from 2059 in steps of 17 for a duration of three minutes. The acute stress induction procedure lasted in total about 8 minutes including instructions. For detailed information about the procedure, see Kaldewaij et al. (2019) and Zhang et al. (2019). Five salivary samples were collected during the acute stress induction procedure using Salivettes collection tubes. Depending on the onset time of stress induction, samples were taken at timepoints −10, 0, +10, +20, and +30 minutes. Samples were analyzed by the Dresden Labservice in Germany using a chemiluminescence immunoassay with high sensitivity (IBL Inc.). As the fifth sample was not obtained for all participants, delta cortisol will be obtained by subtracting the cortisol levels of the pre-stress sample (T = 0) from the peak post-stress sample (T = +20) resulting in a sample of N = 130 in the final analysis.

2.3.3 HEART RATE VARIABILITY

Heart rate recordings were collected during resting-state scans by attaching a finger pulse photoplethysmography to the participant's left index finger. Duration of the scans was approximately 9 minutes. Data was preprocessed for peak detection and corrected for movement by in-house software. Data was manually checked to avoid undetected peaks becoming detected and noise caused by movements was deleted. Next, the root mean square of successive differences (r-MSSD) of HRV was used as time domain variable which is independent of long-term trends and reflects predominantly vagal tone (50, 51). In total a sample of N = 135 was used in the final analysis.

2.3.4 DENTATE GYRUS VOLUME

DG volume was acquired with structural T1-weighted magnetic resonance imaging (MRI). Using a longitudinal processing pipeline provided 12 bilateral hippocampal subfields from which CA1, CA3 and DG were extracted. Volume alterations in these structures are observed in PTSD development (52-55). For detailed procedures, default options and MRI-acquisition parameters see Koch et al. (2020; in press). After quality checks and excluding one outlier ($|Z| > 3.29$) a total sample of N = 102 was included in the analysis.

2.3.5 APFC ACTIVITY

aPFC activity was measured with the approach avoidance (AA) task in combination with fMRI. Participants were asked to move a joystick as fast and accurate as possible to pictures of facial expressions. Approaching happy faces is an automatic tendency together with avoiding angry faces called the congruency effect. Controlling these automatic tendency actions, meaning approaching angry faces and avoiding happy faces (incongruency effect), is reflected by more aPFC activity (18, 56, 57). Investigating the relationship between aPFC activity and PTSD symptoms, left aPFC activity during emotional incongruent versus congruent actions was used as predictor for PTSD symptom development. For more information about the AA task used and MRI acquisition see Kaldewaij et al. (2019). Participants were excluded from analysis when anatomical abnormalities, poor imaging quality, excessive movement during MRI acquisition were found or poor task performance meaning < 10 trials correct. Consequently, the sample for final analysis included N = 130 participants.

2.3.6 AMYGDALA ACTIVITY

Left dorsal amygdala activity was acquired using a shooting task in combination with fMRI. This task is designed to study defensive reactions. Participants were asked to respond on a virtual opponent that could either draw a gun or a phone. Instructions were specifically to shoot as fast and accurate as possible only when the opponent drew a gun. Threat was implemented in the task based on two levels. Low threat (safe trials) meaning when decision making was wrong the participants were shot and high treat meaning being shot in combination with an aversive electric shock. In the time period between the opponent coming up on the screen and the opponents' response, participants need to anticipate on this response. In this period, it is likely that freezing occurred and therefore also called the freeze interval (FI). Investigating the predictive effect on PTSD symptom development, left dorsal amygdala activity during FI with contrast threat > safe was used in the analysis. For detailed information about the task and MRI acquisition see Hashemi et al. (2019). Participants that did not go into the scanner, did not follow task instructions or where data was lost were excluded from analysis resulting in a total sample of N = 99 in the final analysis.

2.3.7 BRAIN NETWORK CONNECTIVITY

Brain network connectivity changes during acute stress were assessed using the same SECPT and MA task described in section 2.3.2. Changes in functional connectivity of resting state networks is defined as difference in connectivity between pre- and post-stress scans. Based on earlier findings, only SN connectivity changes were predictive for PTSD symptom development (42). Therefore, in our analysis we only included SN connectivity changes as predictor for the cluster distribution. For detailed information about MRI acquisition see Zhang et al. (2019). Participants were excluded from analysis due to technical issues, motion artefacts or anatomical abnormalities resulting in a total sample of N = 117 participants included in the final analysis.

2.4 Statistical analysis

2.4.1 SYMPTOM DEVELOPMENT AND TRAUMATIC EXPERIENCES

Development of PCL symptom scores over time was analysed using a mixed effects model preventing listwise deletion of missing data. There is a strong association between the frequency of traumatic events experienced and attenuated mental health outcomes, including PTSD development (58, 59). Because of this strong association, the number of traumatic events experienced (PLES) could explain the variance in PTSD symptom score and was therefore included in the model with dependent variable *PCL score* and independent variables *Time* and *PLES* and interaction *Time*PLES*. To account for between subject variance in the average PCL score and the effects of PLES and Time thereon, we fitted random intercepts for subject *ID*, random slopes for *Time* and *PLES* per subject and random correlations for all random intercepts and slopes.

2.4.2 CLUSTER ANALYSIS

Identifying the different trajectories of stress symptom development over the four time points, a nonparametric K-means cluster analysis was used specifically designed for longitudinal datasets (KmL) (60). Growth curve mixture modelling is a parametric approach to find latent trajectory classes and requires assumptions of trajectories and distributions of observations. Because of these conditions big enough sample sizes are a prerequisite to find the defined trajectories, which is lacking in the current analysis. Modeling trajectories with parametric approaches require too many parameters and therefore the decision was made to drop the parametric assumptions and explore the different trajectories of stress development using a nonparametric approach. KmL has the advantage that it can work with smaller sample sizes because it is not required to define trajectories and distributions of observations a priori. Numbers of clusters was determined looking at multiple criterion: Calinksi and Harabatz (61), Ray and Turi (62) and Davies and Bouldin (63). Remaining sufficient power within the trajectory analysis was achieved by including participants with one missing value in this longitudinal approach. A minimum of three time points is required for modelling a linear pattern, whereas four time points are more ideal to include also quadratic patterns (64). K-means cluster analysis was carried out using R Statistics version 4.0.2 (65) and KmL version 2.4.1 (60). Identifying characteristics of participants within the two clusters, baseline characteristics were compared per cluster (Table 2). For continuous variables, a two-sample t-test was conducted and for categorical variables X^2 tests.

2.4.3 PREDICTOR ANALYSIS

To measure the predictive effect of the physiological and neurological measurements on the clustering, a binomial logistic regression was conducted with dependent variable *clusters*. First, we examined whether neural predictors derived from earlier work on the prospective study was still predictive of the symptom increase between T2 and T2 in this study by conducting separate simple linear regressions. Next, due to high intercorrelation between the variables, separate simple logistic regressions were conducted with the physiological predictor hair cortisol, salivary cortisol and HRV. Same analyses were done for neurological predictors DG volume, aPFC activity, amygdala activity during freezing, and SN connectivity changes. As a follow-up analysis, separate logistic regression analyses were carried out with *cluster* as dependent variable and the neural predictors as independent variables including covariates baseline PCL, baseline PLES, baseline VAS and baseline BDI to see if the effect remains significant. Last, a multiple logistic regression was conducted to see if the predictive effect of the neural predictor remained significant after including the physiological measurements in the model.

Table 1 - Demographics and clinical characteristics of recruits' baseline measurements in the prospective longitudinal study investigating stress symptom development after trauma exposure. Values in the table represent mean and standard deviations of continuous variables, for categorical variables the values represent frequency and percentage. Analysing the difference between clusters, 2-sample t tests were carried out for continuous variables and $\chi 2$ tests for categorical variables. Changes over time were tested with repeated measures ANOVA (within-subject factor: time; between-subjects factor: ID). AUDIT = Alcohol Use Disorders Identification Test; PLES = Police Life Event Scale; PCL-5; PTSD Checklist for DSM-5; BDI = Beck Depression Inventory; PSS = Perceived Stress Scale; VAS = Visual Analogue Scale (sum of three negative items).

Demographics	**Cluster A "Resilient"**		**Cluster B "Increased symptoms"**		
	N = 113		**N = 27**		
	Mean/N	**SD/%**	**Mean/N**	**SD/%**	**P-value**
Sex (females)	27	22,9	6	23,1	1
Age	24.43	5.76	23.81	4.76	0.564
AUDIT - alcohol use; total baseline score	5.07	2.69	5.74	3.65	0.376
Educational level					
High	2	1.8	1	3.7	
Middle	82	72.6	22	81.5	0.964
Low	8	7.1	2	7.4	
Caucasian ancestry	101	89,4	27	100	0.077
CTQ - childhood trauma; total baseline score	30.02	6.35	31.41	4.27	0.646
Previous military experience					
No experience	95	84.0	21	77.8	
Army training, no deployment	9	8.0	5	18.5	0.214
Combat exposed	9	8.0	1	3.7	
PLES - life events; total baseline score	1.34	1.79	2.81	2.43	0.006
PCL-5 - PTSD symptoms; total baseline score	4.06	4.99	9.04	7.33	0.002
BDI - depression; total baseline score	2.16	2.67	3.70	3.44	0.036
PSS - perceived stress; total baseline score	14.15	7.03	16.89	7.25	0.084
VAS - negative affect; total baseline score	7.07	3.81	9.19	3.44	0.008

	Baseline		**Wave 2**		**Wave 3**		**Wave 4**		**Main effect of Time**		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	***F***	***df***	***p***
AUDIT	5.20	2.89	6.20	3.12	n/a	n/a	n/a	n/a	64.14	370.73	<.001
BDI	2.46	2.89	2.78	3.17	4.49	4.11	4.47	3.98	116.45	769.22	<.001
PCL-5	5.02	5.83	6.44	9.65	4.30	6.83	4.65	7.80	9.33	658.44	0.002
PLES	1.62	2.01	5.89	3.57	7.94	4.29	7.04	4.20	229.4	897.54	<.001

	Baseline		**Int. 1**		**Int. 2**		**Wave 2**		**Wave 3**		**Wave 4**		**Main effect of Time**		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	***F***	***Df***	***P***
PSS	14.68	7.13	16.13	6.81	17.17	8.00	15.19	7.97	15.53	8.54	14.38	8.38	7.4633	1590.6	0.006
VAS	7.48	3.83	7.15	3.96	8.50	4.48	8.30	4.89	6.90	4.64	6.10	3.70	12.701	1589.2	<.001

3 RESULTS

3.1 Stress symptom development and relation to trauma exposure

Investigating the change in PTSD symptoms over time controlling for the number of traumatic events experienced that seems to be a main explanatory variable of PTSD symptom development showed a significant effect of *Time* [$F_{1,\ 138.26} = 6.888$; $p = 0.010$], no significant effect of *PLES* [$F_{1,228.50} = 0.0371$; $p = 0.847$] and no interaction effect [$F_{1,286.87} = 0.0142$; $p = 0.905$] on PCL symptom score. In other words, the PTSD symptoms seemed to change significantly over time, but the number of traumatic events could not explain the overall variance in PTSD symptom scores or the changes in PTSD symptom score over time. However, it remains still unclear on which timepoints the PTSD symptoms are significantly increasing or decreasing.

Follow-up analysis using paired sample t-tests and FDR-correcting for multiple comparisons showed a significant decrease from T2 (M = 6.44; SD = 9.65) to T3 (M = 4.30; SD = 6.83); [$t_{132} = 3.62$; $p = .002$], and a significant decrease between T2 and T4 [$t_{112} = 2.52$; $p = 0.039$]. There were no significant differences found between the other timepoints (Figure 2). It seems that the overall pattern of the population shows a slight increase in PTSD symptoms right after trauma exposure and a significant decrease in symptoms when working as fully trained police officer in the field. However, individual patterns of PTSD symptom development seem to be very heterogeneous (Figure 3A). These patterns will be investigated further with a cluster analysis trying to identify trajectories of PTSD symptom development.

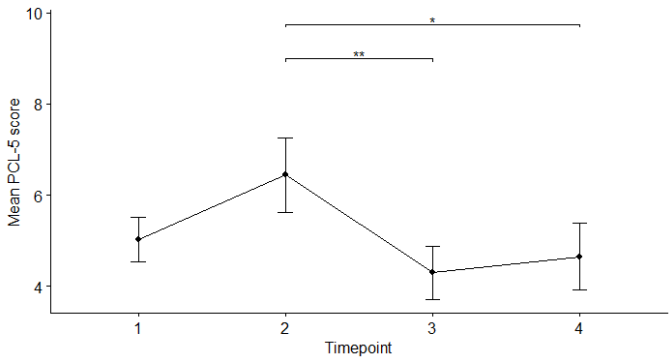

Figure 2 – Average PTSD symptom scores (PCL-5) for the first four timepoints of the prospective longitudinal study in Dutch police officers (N = 144).

* = p < 0.05; ** = p < .01

3.2 Cluster description

Optimal number of clusters is determined by the maximum votes between the Calinksi and Harabatz criterion (61), Ray and Turi criterion (62) and Davies and Bouldin criterion (63) that all three agreed that two clusters were the optimal. This study of longitudinal patterns of stress development identified two distinct groups with a k-means cluster analysis on longitudinal data: *resilience* (80%) and *increased symptoms* (20%; Figure 3A). Studying the effect of clusters and time on the PCL score, a mixed effects model was conducted with dependent variable *PCL score*, independent variable *clusters* and *Time* and interaction *clusters*Time.* To account for between subject variance in *PCL score* and the effects of *Time* thereon, we

fitted random intercepts for subject *ID,* random slopes for *Time* and random correlations for all random intercepts and slopes. Results showed a significant effect of *Time* [$F_{1,132.24} = 4.1158$; $p = 0.044$] and *clusters* [$F_{1,135.41} = 18.6032$; $p < .001$] but no interaction effect of *clusters*Time* [$F_{1,132.35} = 2.1792$; $p = 0.142$]. In other words, the level of PTSD symptoms is in one cluster higher compared to the other, over time the PTSD symptom scores change but this change over time is not found for the clusters separately. Baseline characteristics per cluster are described in Table 1. Compared with individuals in in the resilient group, individuals in the increased symptom group experienced more different traumatic events (PLES), experienced more PTSD (PCL-5) and stress symptoms (PSS) and had more negative (VAS) and depressed feelings (BDI). There were no differences in gender, age, alcohol use (AUDIT), education, ethnicity, childhood trauma (CTQ) or military deployment (Table 1).

Follow up analyses were carried out studying the changes of PTSD symptoms over time for each cluster using a mixed effects model with dependent variable *PCL,* independent variable *Time,* random intercepts for subject *ID* and random slopes for *Time* per subject. In the resilient group, there was a significant linear [$F_{1,106.8} = 7.2038$; $p = 0.008$] but no quadratic [$F_{1,225.84} = 2.1648$; $p = 0.143$] effect of *Time* on PCL symptom score. For the increased symptom score group there was a quadratic effect [$F_{1,54.153} = 13.173$; $p < .001$] and no linear effect [$F_{1,25.543} = 0.1801$; $p = 0.675$] of *Time* on PCL symptom score. Despite the non-significant *cluster*Time* interaction, these results suggests that the patterns of PTSD symptom development over time is significantly different between the clusters.

To further investigate the temporal development of PTSD symptoms, paired-samples t-tests were conducted to compare the differences in symptom scores between time points within each group. Results were FDR-corrected for multiple comparisons. The resilience group reported relatively low symptoms scores over all four timepoints with a significant decrease in PTSD symptoms between T1 (M = 4.0; SD = 4.960) and T2 (M = 3.2; SD = 4.440); [$t_{111} = 2.69$, $p = 0.019$], T1 and T3 (M = 2.3; SD = 2.564); [$t_{107} = -3.45$, $p = 0.003$] and T1 and T4 (M = 2.4; SD = 3.106); [$t_{92} = 2.96$, $p = 0.004$] (Figure 3B). For the increased symptoms group there was a significant increase in PTSD symptom scores between T1 (M= 9.8; SD= 7.419) and T2 (M= 23.5; SD=11.8); [t_{25}=--5.48, $p < .01$], a significant decrease in PTSD symptoms between T2 and T3 (M = 13.6; SD = 11.154); [$t_{25} = 4.26$, $p < .01$] and a significant difference in PTSD symptom score between T2 and T4 (M = 15.2; SD = 11.6); [$t_{20} = 2.70$, $p = 0.028$] (Figure 3B). The differences in PCL-5 score between groups on different timepoints are carried out with an independent sample t-test. Results of this analysis showed a significant difference in PCL-5 scores between all four timepoints [T1: $t_{39.478} = -3.866$, $p < .001$; T2: $t_{52.105} = -14.966$, $p < .001$; T3: $t_{40.892} = -9.033$, $p < .001$; T4: $t_{26.589} = -5.464$, $p < .001$] (Figure 3A). This suggests that compared to the resilient group, the increased symptoms group show higher PTSD symptom scores on all four timepoints (Figure 3A).

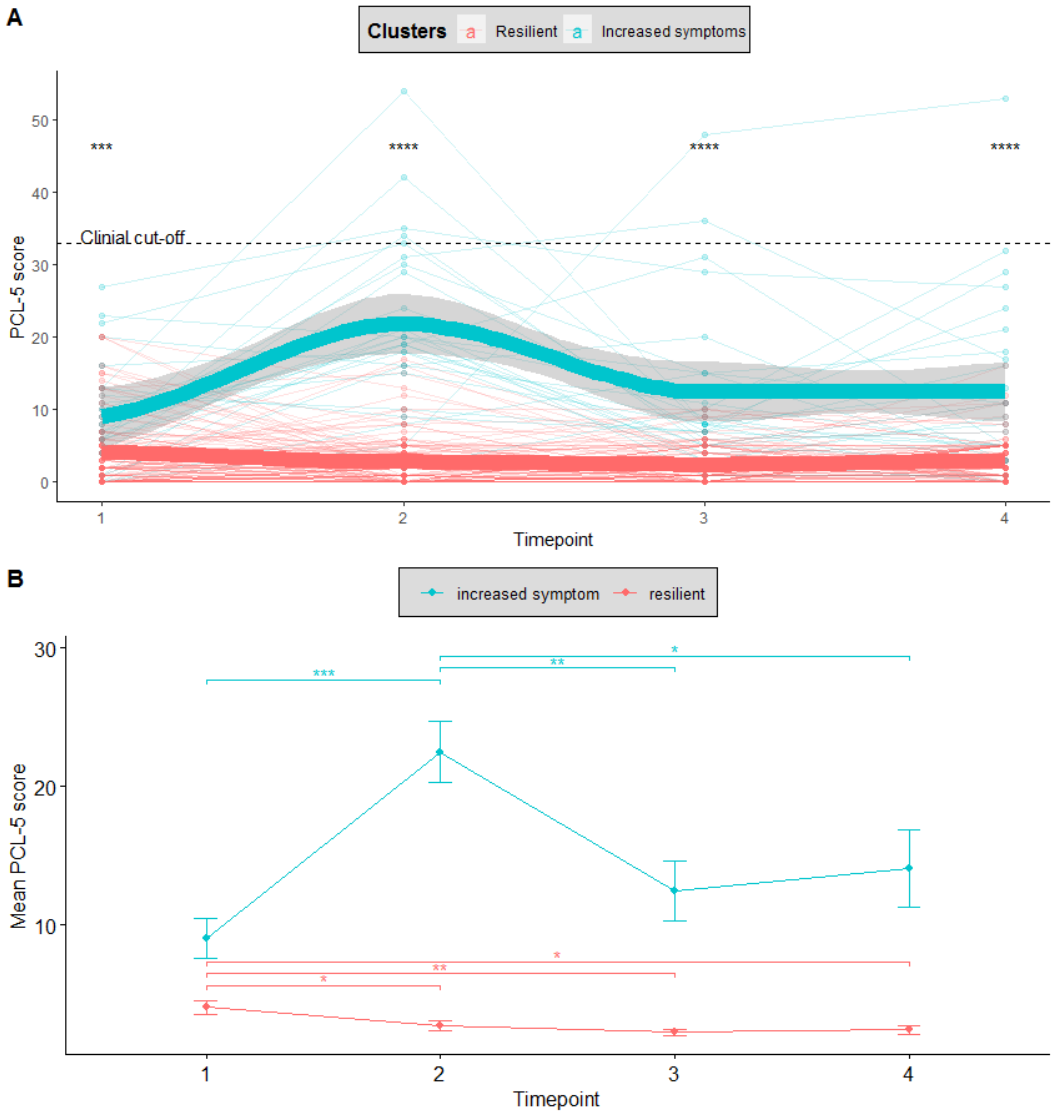

Figure 3 – **A.** Individual and mean stress symptom trajectories from a K-means cluster analysis on longitudinal data (KmL) based on PCL-5 scores for the first four timepoints of a prospective longitudinal study. Two distinct trajectories are identified: resilience group (red, n = 113; 80%) and increased symptoms group (blue, n = 27; 20%). **B.** Mean PTSD symptom score and SD of each cluster per timepoint.

* = p < 0.05; ** = p < .01; *** = p < .001, **** = p < .0001

One reason that could explain the differences in clusters is the number of traumatic events police officers experienced (58, 59). To control for the number of traumatic events experienced (PLES), the same mixed model was carried out studying the effects of *cluster*, *Time* and *cluster*Time* on *PCL score* including PLES as covariate. Results showed a significant effect of *clusters* [$F_{1,132.319}$ = 18.328; p < .001], a marginally significant effect of *Time* [$F_{1,126.025}$ = 3.1082; p = 0.080], a trend effect of *PLES* [$F_{1,87.041}$ = 3.9073; p = 0.051] and no interaction effect of *clusters*Time* [$F_{1,122.993}$ = 2.632; p = 0.107]. This suggests that the number of different traumatic events experienced may account for the cluster distribution, however cautious interpretation is required because this result was not significant.

3.3 Prediction of clusters

To investigate potential neurobiological mechanisms as origin for the observed symptom development trajectories we included neural substrates and physiological measurements in a logistic regression model. First, we explored whether our key neural predictors derived from earlier work on T1 and T2 were still predictive of the symptom increase in this study. Analyses were reproduced in this cohort using simple linear regression analyses. Results showed that amygdala activity during the freeze interval predicted change in PCL score between T2 and T1 (ΔPCL_{T2-T1}), also after FDR-correction for multiple comparisons (Table S1). Follow-up analysis using multiple linear regression controlling for the same variables used in earlier work on T1 and T2 showed again only a significant predictor effect of the amygdala for ΔPCL_{T2-T1} with the same results after FDR-correction for multiple comparisons (Table S1). In other words, higher amygdala activity predicted higher vulnerability of PTSD symptom development from T1 to T2.

Next, to investigate the predictive effect of neural- and physiological variables on the cluster distribution separate simple logistic regression analyses were carried out with dependent variable *clusters* and independent variable the predictor. These models were carried out separately because of high intercorrelation among the variables. Results showed only a predictive effect of amygdala activity during the freeze interval on the clustering. Caution of the interpretation of these results is warranted because the effect did not hold after FDR-correcting for multiple comparisons (Table 2).

The predictive effects of the neural baseline measurements are further investigated by controlling for variables that showed a significant effect between the two clusters. Results showed only a significant predictor effect of amygdala during freezing on the cluster distribution ($p = 0.020$; Table 2), however caution of interpretation is still warranted because there was no significant effect after FDR-correction for multiple comparisons ($p = 0.80$; Table 2).

Despite the non-significant results after correction for multiple comparisons, the results do show a trend towards significance. Therefore, further analyses were carried out to investigate the origin of these effects. Investigating if the amygdala effect still holds when somatic predictors were included showed still a significant predictor effect of amygdala on the cluster distribution ($p = 0.025$; Table 2). These results suggests that higher amygdala activity at baseline predicts a higher risk of belonging to the increased symptom trajectory above and beyond other somatic predictors.

Previous results show that the amygdala activity during defensive responses is able to predict the trajectories of PTSD symptom development, however It remains still unclear if the amygdala activity predicts the cluster distribution based on the significant increase of PCL score between T1 and T2 (Table S1) or that the effect is also there for other time points. First, baseline amygdala activity between clusters was investigated using an independent sample t-test showing a marginally significant lower baseline amygdala activity in the resilience group compared to the increased symptom group (Table 3). Next, the effect of amygdala on PTSD symptom development on other timepoints was investigated using separate linear regressions with independent variable amygdala activity. Results showed only a predictive effect on

dependent variable ΔPCL_{T2-T1} (Table 3). In other words, increased amygdala activity at baseline was associated with a higher increase in PTSD symptoms from T1 to T2 (Figure 4A). Looking at the predictive effect of baseline amygdala activation on ΔPCL_{T3-T2} showed a marginally significant effect (p = 0.079; Table 3). However, caution interpretation is warranted because this effect is probably driven by outliers. Carrying out a spearman-rank correlation allowed to study the monotonic relationship between the amygdala during freezing and the change in PTSD symptoms between T2 and T3 and showed no significant relationship (p = 0.194). There is some evidence that increased amygdala may also be related to a decrease in PTSD symptoms between T2 and T3. However cautious interpretation is warranted because this effect was not significant and probably driven by outliers (Figure 4B).

Table 2 - Overview of the predictive effect of neural- and physiological predictors on the clusters (resilient- and increased symptoms group) using logistic regression using simple logistic regression and follow-up analysis controlling for significant baseline differences between the clusters in a mixed effects logistic regression. DV = dependent variable; IV = independent variable; CV = covariate; FI = freeze interval; DG = dentate gyrus; aPFC = anterior prefrontal cortex; SN = salience network; HCC = hair cortisol concentration; HRV = heart rate variability; bPCL = baseline PTSD checklist for DSM-5; bPLES = baseline police life events scale, bVAS = baseline visual analogue scale; bBDI = baseline beck depression index.

Simple logistic regression						
DV	**IV**	**CV**	***ß***	***Z***	***P***	P_{FDR}
Clusters	Amygdala (FI)	n/a	2.387	1.983	0.047	0.189
	DG	n/a	-91.788	-1.424	0.154	0.206
	aPFC	n/a	-2.531	-1.447	0.148	0.206
	SN	n/a	-0.301	-0.598	0.550	0.550
	Salivary cortisol	n/a	0.175	0.209	0.85	n/a
	HCC	n/a	-0.243	-0.474	0.635	n/a
	HRV	n/a	-0.066	-0.078	0.938	n/a
Mixed effects logistic regression						
Clusters	Amygdala (FI)	bPCL, bPLES, bVAS, bBDI	3.779	2.328	0.020	0.080
	DG	bPCL, bPLES, bVAS, bBDI	-121.761	-1.444	0.149	0.298
	aPFC	bPCL, bPLES, bVAS, bBDI	-2.492	-1.097	0.273	0.364
	SN	bPCL, bPLES, bVAS, bBDI	-0.323	-0.556	0.578	0.578
Clusters	Amygdala Salivary cortisol HCC HRV	n/a	7.455 2.145 -1.472 4.295	2.234 0.786 -1.081 1.547	0.025 0.432 0.280 0.122	n/a

Table 3 - Overview of the differences in baseline amygdala activity during the freeze interval (FI) between the resilient and increased symptom trajectories investigated with independent sample t-test. Using linear regression models the predictive effect of amygdala activity on the development of PTSD symptoms (PCL) on different timepoints was studied. Change in PCL score between two timepoints is indicated by ΔPCL.

Predictor		**Cluster A "Resilient"**		**Cluster B "Increased symptoms"**		
		N = 113		**N = 27**		
		Mean	**SD**	**Mean**	**SD**	**P-value**
Amygdala (FI)		0.012	0.202	0.112	0.210	0.053
DV	**IV**	***R²***	***ß***	***t***	***df***	**P-value**
ΔPCL_{T2-T1}	Amygdala (FI)	0.072	0.242	2.722	96	0.008
ΔPCL_{T3-T1}		0.005	0.123	1.259	94	0.211
ΔPCL_{T4-T1}		-0.008	0.071	0.609	81	0.544
ΔPCL_{T3-T2}		0.033	-0.106	-1.779	93	0.079
ΔPCL_{T4-T3}		-0.012	-0.027	-0.203	78	0.840
PCL_T1		0.002	0.045	0.419	97	0.676
PCL_T2		0.064	0.245	2.570	96	0.012
PCL_T3		0.034	0.185	1.824	94	0.071
PCL_T4		0.023	0.165	1.389	81	0.169

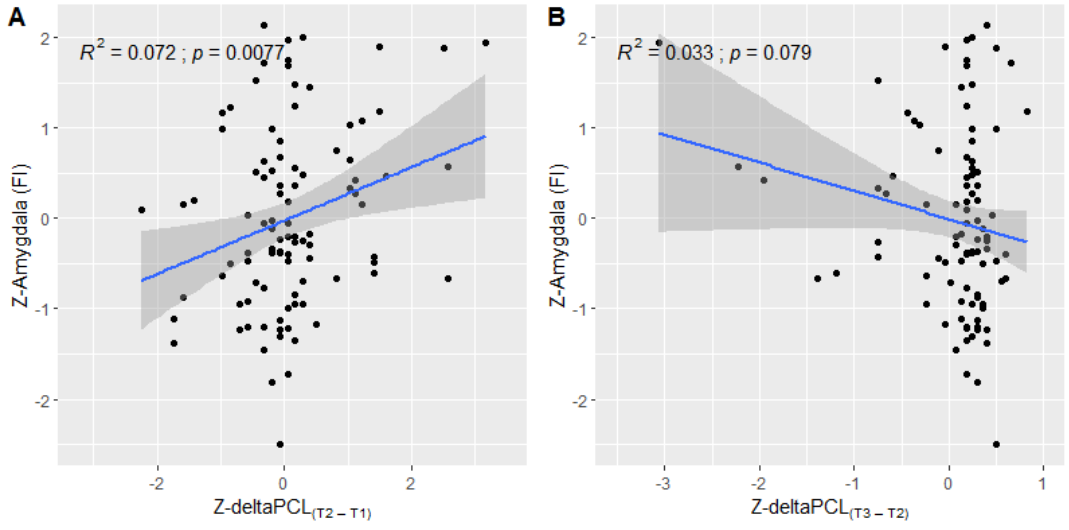

Figure 4 – A. Linear regression analysis of the predictive effect of baseline amygdala activity during defensive responses on ΔPCL_{T2-T1} showing a positive correlation suggesting higher amygdala activity is associated with higher PTSD symptoms on T2 compared to T1. **B.** Linear regression analysis of the same amygdala activity on ΔPCL_{T3-T2} showing a negative correlation suggesting higher amygdala activity is associated with lower PTSD symptoms on T3 compared to T2.

4 DISCUSSION

In this prospective study, trajectories of stress symptom development are identified in Dutch police recruits exposed to traumatic events. Two distinct groups can be identified looking at longitudinal stress symptom development. The resilient group (80%) showed relatively low stress symptoms over time, whereas the increased symptom group (20%) showed higher baseline symptom scores with a strong temporal increase right after trauma exposure that decreases again to a higher baseline level compared to the resilience group. Neural- and physiological baseline measurements are investigated that may function as a marker of stress resilience or vulnerability of PTSD development. Increased baseline amygdala activity during freezing predicted higher vulnerability of belonging to the increased symptom group above and beyond potential influences of other somatic predictors.

In line with our first hypothesis, Dutch police recruits can be roughly divided into two groups based on stress symptom development over time. Trajectories were labelled as resilient and increased symptoms based on the overall symptom profile. Nevertheless, this does not mean that the resilience group do not show (temporal) fluctuations in PTSD symptom development. On average, the resilience group show low symptom scores across all timepoints. However, some individual trajectories within the resilience group showed a temporal increase of PTSD symptoms at one of the time points (Figure 3A). After this short increase, the symptom scores rapidly declined back to low baseline levels so that on average they reported low symptom scores over time. Because baseline symptom scores of the increased symptom group are higher compared to the resilience group, this suggests that vulnerability can be determined already before first trauma exposure. Besides, subjects that belonged to the increased symptom group, felt more depressed, perceived more stress and experienced more negative emotions before trauma exposure. Also, these subjects experienced before trauma exposure more different types of events compared to the resilient group which is in line with the literature (66-68). Our findings support the idea that different patterns of stress symptom development can be identified. The ability to detect vulnerability of stress symptom development already before trauma exposure is important for applying early interventions. This is also relevant for educational purposes focussing more on individual guidance, support and training to improve resilience.

Longitudinal studies done by Galatzer-Levy (2018) and Bonanno (2014) show four different patterns of stress symptom development. This is contrary to our findings showing only two trajectories. One explanation for these discrepancy in results is the different cluster analysis used (parametric vs. non-parametric approach). Another reason could be the differences in sample size. Comparable studies that used sample sizes with 200+ participants, were able to identify four different trajectories (67, 69). Another comparable longitudinal study with sample size of 178 participants could identify only three different trajectories (70). Taken these results together supports the importance of a big enough sample size for the number of trajectories that can be identified. One other important thing to note is the relatively healthy

sample in this study that was confirmed by earlier work done in the same prospective longitudinal study (39-42). Because only a small percentage of police officers show clinically significant PTSD symptoms, we are limited to generalize our findings to full-blown PTSD development. This could at least explain why the 'chronic' group was not identified in our cluster analysis. In line with the aforementioned longitudinal studies, our study confirms the idea that only a small percentage of police recruits develop PTSD symptoms (1, 7, 71). This suggests that despite being at higher risk of trauma exposure, police officers tend to be resilient to stressors. Several factors could be of impact for being more resilient such as selection criteria used when hiring new recruits (72) or specific training to cope with stressful events (73). Another explanation is the use of self-reported questionnaires that result in underreporting of symptom experience due to the police culture that promotes more socially desirable responses (74). This contradicts early beliefs that exposure to traumatic exposure goes together with disruption of normal functioning and therefore resilience must be rare (75). More recent studies are however also in line with our results showing that resilience is indeed more common and the majority of a population exposed to stressors are coping well (7, 12, 71, 76).

In line with our second hypothesis, we found that increased amygdala activity during a freezing state is predictive for the cluster distribution. In other words, amygdala activity during defensive freezing before trauma exposure is able to predict if people are more vulnerable for PTSD development or seem to be more resilient. Previous studies looked at amygdala activity during a passive viewing task showing that baseline amygdala reactivity predicts stress symptom increases (77, 78). Police officers work in a dynamic environment and often need to respond to a threatening situation by activating defensive reactions. Amygdala activation is the start of activating these processes where information is projected to multiple brain areas including the periaqueductal gray (PAG). As a result, defensive freezing will occur to prepare for action in distal threat whereas at proximal threat the amygdala induces fight or flight reactions. There are only a few studies that investigate the amygdala activity during this freezing interval, suggesting that amygdala activity during freezing is a predictor for PTSD vulnerability right after trauma exposure (41). Our study adds to this information that baseline amygdala activity during defensive reactions also predict vulnerability of PTSD over a longer time period, because it can predict different trajectories of stress symptom development. There are also indications that amygdala activity increases after severe trauma exposure suggesting a complex interaction between predisposition and the effects of trauma exposure which was not studied in this project (40, 79). Future research could focus on this complex mechanism studying amygdala activity changes over time. Our findings support the idea that the amygdala plays a crucial role in PTSD symptom development and suggests that reducing amygdala activity during training or prevention methods may improve resilience, aiming to prevent PTSD development.

Contradictory to our hypotheses, no other neural and physiological baseline measurements were predictive for the cluster distribution. These results suggest that there is no longitudinal effect of the neural predictors on PTSD symptom development. However, a reason that could explain why there is no predictive

effect found for the neural substrates is the differences in sample size. Compared to earlier work done on T1 and T2 that showed a predictive effect of lower DG volume, lower aPFC activity and increased SN connectivity changes on PTSD symptom development (39, 40, 42), we could not replicate the same effects in our cohort (Table S1). Despite the non-significant results of the neural predictors, negative correlations were found looking at the predictor effects of DG, aPFC and SN connectivity on changes in PTSD symptoms before and after trauma exposure. In other words, a real small effect of lower DG volume, less aPFC activity and less SN connectivity was found in relationship with higher vulnerability of PTSD symptom development (Table S1). Within this follow-up study we lost participants due to changed personal information which is the main reason for the lower sample size. It is recommended for future longitudinal studies to collect personal information that is unlikely to change. An explanation for finding no predictive effect of physiological predictors on the trajectories, may be the relatively healthy sample targeted in this study. These predictors may become apparent in more severe PTSD cases which could be an interesting topic for future studies.

Finding no predictive effect of cortisol on vulnerability of PTSD development contradicts the results from other longitudinal studies suggesting that lower cortisol responses during acute stress predict vulnerability of distress later in life when exposed to multiple stressors (27, 69). On the contrary, there are also studies that are in line with our results showing no effect of salivary cortisol on PTSD development (42, 80, 81). A meta-analysis on the relationship between HCC and stress symptom development showed reduced HCC in patients suffering from anxiety disorders such as PTSD. However, no association was found between HCC and self-reported perceived stress (82). The discrepancy of results may arise from timing of assessment after trauma exposure. Some studies measure once a year for over four years (69), twice in 12 months (27), twice in 16 months (42) or four measurements within six years in our study. Discrepancy could also arise due to different analytical approaches using continuous modelling or subtyping. Diversity in sample characteristics using police recruits (42, 69) or combat soldiers (27) could also explain this discrepancy. Future studies investigating the predictive effect of cortisol on symptom development should take these differences into account.

No predictive effect was found of resting state HRV on the cluster distribution which is against our hypothesis. A meta-analysis showed that lower HRV is associated with development of anxiety disorders, including PTSD (83). In line with this meta-analysis a pre- and post-deployment study in military showed similar results that pre-deployment HRV predicted post-deployment PTSD symptoms, however this relationship was only in context of higher pre-deployment PCL scores (23). An explanation why the results are contradictory could be different use of HRV metrics and norms. In our study the RMSSD was used whereas other studies use other metrices such as SDNN or NN50 (84, 85). All these metrices seem to be impacted by the PNS reflecting beat-to-beat changes (85), however the way HRV is calculated is differently and therefore could lead to contrary results. Another reason why this relationship was not found may be because the police officers already started their training at the police academy. This training could have an

effect on their physiological response to stress (73). Note that this is not specifically tested in our study and should therefore be taken into account in future research.

Longitudinal effects of stress development are extensively studied. However, the large majority of these studies start measuring after trauma exposure and therefore lack baseline measurements where participants are not exposed to trauma yet. Physiological measures such as cortisol (69), emotion (67) and anxiety (86) seem to be predictors for longitudinal stress symptom development, but the role of neural measurements as possible predictors is still unknown. Importantly, PTSD symptom development is very heterogeneous which requires a more dimensional approach looking at individual patterns of stress symptoms development after trauma exposure. Using a trajectory approach has the advantage to visualize subtle individual changes in stress symptom development. With the two extra follow-up measures, symptom development was measured for a longer time period after trauma exposure to investigate prolonged PTSD development. Due to the baseline measurements, we can suggest that vulnerability of stress symptom development may already be determined before first trauma exposure. Knowing if a person has a higher risk for stress symptom development is valuable information for both the National Police as well as for the persons themselves. This prior knowledge can be used to change the focus from a general group approach to an individual level. Spending more individual attention, guidance and support in coping and dealing with stress and their symptoms could eventually prevent the development of full-blown PTSD. The results of this study give insights in different trajectories of stress development, what explanatory variables are for these different trajectories and on which characteristics these trajectories are distributed. Increased amygdala activity before trauma exposure seems to be a predictor for higher vulnerability of PTSD symptom development. Resilience may be improved when amygdala activity can be reduced before trauma exposure. Applying individual neurofeedback training may be a useful method aiming to prevent PTSD symptom development.

People experience in their life at least one traumatic event and the severity of these events can differ. The traumatic event that has the most impact on a person's life is called core trauma. Core trauma was in this prospective longitudinal study assessed via a Clinician-Administered PTSD scale for DSM-5 (CAPS) interview, but this was done only at T2. It is unknown if the participants were exposed to a new core trauma after T2. In this study, the general development of stress symptoms after experiencing trauma is studied without taking into account core trauma exposure. This is in line with previous studies done in American police officers (69, 70). One of the main reasons to not include it in this study is the sample size. A cohort of 140 recruits is already quite small especially for parametric cluster analysis identifying four expected groups. PTSD development within a population is often divided in 75% resilient individuals, 11.25% recovery, 7.5% chronic and 6.25% delayed onset (71). Based on our sample size of 140, the delayed onset group would include a maximum of 9 participants which does not reach the requirement of having a minimum of 10 cases per expected group (87). It is hard to make proper estimations of the right sample size which depends on the number of parameters, amount of missing data, distribution of the variables

and the relationship with effect sizes (64) and often the rule of thumbs used in these models lead to over- or underestimation of the required sample size (88, 89). Insufficient sample sizes result in convergence issues and disability of detecting small groups such as the delayed onset group in this study. Dealing with this issue by using a non-parametric cluster analysis resolved this problem by dropping the parametric assumptions. Therefore, this analysis became more exploratory assuming that the different trajectories of PTSD symptom development have each a unique time course. It is recommended for future longitudinal studies on PTSD development to use bigger sample sizes and to assess core traumatic event for each timepoint to gain better insights in individual changes of symptom scores between the timepoints.

Another limitation is the number of measurements within the time frame. Only four measurements are collected over approximately 6 years after starting the police academy. Therefore, one can discuss if the temporal increase after trauma exposure can be characterized as recovery described in other studies (1, 71, 76). Recovery is characterized as a temporal dysfunction due to psychopathology such as increased depression or PTSD symptoms that lasts for at least several months before symptoms decrease again (76). Resilience is not just the absence of psychopathology but can involve temporal perturbation of normal functioning that overall is only present for a couple of weeks (76). Due to the low sampling rate between T2 and T3, it is not clear when the decrease in symptom score started and when it returned back to baseline. This is one reason why the recovery group cannot be distinguished from the resilience group in this study. Also, measuring with self-reported questionnaires could result in reporting higher PTSD symptom scores for just that time window without reflecting the overall symptoms. For future research it is therefore recommended to use a higher sampling rate especially after trauma exposure. A higher sampling rate could also be beneficial for characterizing the delayed onset group or chronic trajectories. From the individual trajectories only a small number of participants seem to show these patterns but were not identified as separate groups in the clustering analysis.

PTSD is a highly heterogeneous psychopathology at all levels, from symptomatology to molecular mechanisms. Using the k-means cluster analysis on longitudinal data (KmL) trajectories of PTSD symptom development were identified. A cluster approach is often a more exploratory analysis because it really depends on the type of data that is used and often the groups do not converge with other studies (90). Studying which baseline measurements are predictors for these trajectories, only a few variables were included in the analysis. The longitudinal prospective study has much more neural-, physiological and psychological measurements that may be possible predictors for vulnerability of trauma susceptibility. Instead of mapping and clustering just the PTSD symptoms, stratification methods can be used as basis of mapping biological mechanisms and cognitive domains within a population (91). Such model has been insightful in studies investigating i.e., stress symptom development after childhood trauma (92) whereas it is yet not used for PTSD symptom development in risk professions such as police officers. A follow-up study could focus on using these methods on the same dataset, to investigate possible predictors for longitudinal PTSD development including all possible baseline measurements.

In summary, this study focussed on identifying different patterns of PTSD symptoms in Dutch police recruits, finding resilient and increased symptoms trajectories. We showed evidence that amygdala activity during defensive freezing before trauma exposure was able to predict PTSD symptom trajectories above and beyond other physiological and psychological predictors, suggesting that already before trauma exposure it can be determined whether people are more vulnerable for PTSD symptom development after PTE exposure. Knowing this in advance is valuable to apply early interventions as soon as possible and to focus more on individual support and guidance. This may improve resilience aiming for prevention of full-blown PTSD development.

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6 SUPPLEMENTARY

6.1 Supplementary table

Table S1. Overview of the predictive effect of neural substrates on the change in PCL score between T1 and T2 using simple linear regression. Follow-up analysis included to control for variables in the mode using multiple linear regression. Controlling variables were chosen based on previous studies (39-42). DV = dependent variable; IV = independent variable; CV = controlling variable; DG = dentate gyrus; FI = freeze interval; aPFC = anterior prefrontal cortex; SN = salience network; bPCL = baseline PTSD checklist for DSM-5; bPLES = baseline police life events scale; CAPS = clinician-administered post-traumatic stress.

Simple linear regression								
DV	**IV**	**CV**	R^2	*df*	*ß*	*t*	*P*	P_{FDR}
ΔPCL_{T2-T1}	DG		-0.005	99	-2.927	-0.678	0.499	0.499
	Amygdala (FI)		0.062	96	0.207	2.722	0.008	0.031
	aPFC		0.002	127	-0.123	-1.102	0.272	0.363
	SN		0.0009	115	-0.054	-1.415	0.160	0.319
Multiple linear regression								
ΔPCL_{T2-T1}	DG	Gender, Age, bPCL, CA1, CA3	0.009	94	-8.974	-1.660	0.100	0.170
	Amygdala (FI)	bPCL, bPLES	0.145	94	0.211	2.905	0.008	0.031
	aPFC	bPCL, bPLES, $\Delta PLES_{T2-T1}$	0.207	123	-0.144	-1.379	0.170	0.170
	SN	bPCL, bPLES, CAPS, ΔPSS_{T2-T1}	0.189	107	-0.003	-0.619	0.160	0.170

6.2 Additional questionnaires

6.2.1 VISUAL ANALOGUE SCALE (VAS)

Method trying to measure an attitude or characteristic within a continuum of values (93). In this study 16 items were measured on a 9-point Likert-scale ranging from 0 (absent) to 9 (extreme/incapacitating). Participants were asked to answer the questions based on their experience over the last two months. VAS scores in this study were gained by the sum of the first three negative scores. Internal consistency of negative affect scores (VAS) was high (Cronbach's alpha = 0.829).

6.2.2 PERCEIVED STRESS SCALE (PSS)

Assessing perception of stress in one's life (94). Participants were asked to answer the questions based on their feelings and thoughts over the last two months. Questionnaire consisted of 14 items on a 5-point Likert-scale ranging from 0 (never) to 5 (very often). Scores were obtained by first reversing the seven positively stated items and then sum across all 14 item scores. There are no cut-off scores because the PSS is not a diagnostic tool. Internal consistency of PSS scores was high as indicated by Cronbach's alpha of 0.861.

6.2.3 CHILDHOOD TRAUMA QUESTIONNAIRE (CTQ)

Self-report questionnaire developed to assess traumatic experiences in childhood (95). Focusses specifically on abuse, physical and emotional neglect. Questionnaire consists of 70-items on a 5-point Likert-scale ranging from 1 (never true) to 5 (very often true). Items are divided over four factors: physical and emotional abuse, emotional neglect, sexual abuse and physical neglect. After reversing the scores of seven items the sum of the total score per factor resulted in the total CTQ score.

6.2.4 BECK DEPRESSION INVENTORY (BDI)

21-item questionnaire designed to assess severity of depression in adolescents and adults (96). Questions are based on the feelings of individuals over a one-week time period. Total BDI score is calculated by the sum score of each individual item. The depression scores (BDI) had a good internal consistency as indicated by Cronbach's alpha of 0.785.

6.2.5 ALCOHOL USE DISORDERS IDENTIFICAITON TEST (AUDIT)

10-item questionnaire assessing alcohol consumption and implications for the subject's health and wellbeing. Internal consistency of alcohol consumption scores (AUDIT) was high (Cronbach's alpha = 0.866).