Identification of biopsychological trait markers in functional neurological disorders

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Abstract

Stress is a well-known risk factor to develop a functional neurological disorder, a frequent neuropsychiatric medical condition in which patients experience a variety of disabling neurological symptoms. Only little is known about biological stress regulation, and how it interacts with predisposing biological and psychosocial risk factors. Dysregulation of the hypothalamic-pituitary-adrenal axis in patients with functional neurological disorders has been postulated but its relationship to preceding psychological trauma and brain anatomical changes remains to be elucidated. We set out to study the hypothalamic-pituitary-adrenal axis analysing the cortisol awakening response and diurnal baseline cortisol in 86 patients with mixed functional neurological symptoms compared to 76 healthy controls. We then examined the association between cortisol regulation and the severity and duration of traumatic life events. Finally, we analysed volumetric brain alterations in brain regions particularly sensitive to psychosocial stress, acting on the assumption of the neurotoxic effect of prolonged cortisol exposure. Overall, patients had a significantly flatter cortisol awakening response ($P < 0.001$) and reported longer ($P = 0.01$) and more severe ($P < 0.001$) emotional neglect as compared to healthy controls. Moreover, volumes of the bilateral amygdala and hippocampus were found to be reduced in patients. Using a partial least squares correlation, we found that in patients, emotional neglect plays a role in the multivariate pattern between trauma history and hypothalamic-pituitary-adrenal axis dysfunction, whilst cortisol did not relate to reduced brain volumes. This suggests that psychological stress acts as a precipitating psychosocial risk factor, whereas a reduced brain volume rather represents a biological predisposing trait marker for the disorder. Contrarily, an inverse relationship between brain volume and cortisol was found in healthy controls, representing a potential neurotoxic effect of cortisol. These findings support the theory of reduced subcortical volumes representing a predisposing trait factor in functional neurological disorders, rather than a state effect of the

illness. In summary, this study supports a stress-diathesis model for functional neurological disorders and showed an association between different attributes of trauma history and abnormalities in hypothalamus-pituitary-adrenal axis function. Moreover, we suggest that reduced hippocampal- and amygdalar volumes represent a biological 'trait marker' for functional neurological disorder patients, which might contribute to a reduced resilience to stress.

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Abbreviations: AAL = Automatic anatomic labelling (atlas); AUC = Area-under-the-curve; BDI = Beck's depression inventory; CAR = Cortisol awakening response; CTQ = Childhood trauma questionnaire; DBC = Diurnal baseline cortisol; DBCC = Diurnal baseline cortisol concentration; FDR = False-discovery rate; FEW = Family-wise error; FND = Functional Neurological Disorders; HC = Healthy controls; HPA = hypothalamus-pituitary-adrenal (axis); PACC = Post-awakening cortisol concentration; PLSC = Partial least squares correlation; ROI = Region-of-interest; STAI = State-trait anxiety inventory; TEC = Traumatic experiences checklist; TIV = Total intracranial volume; SVD = Single value decomposition

Introduction

Functional neurological disorders (FNDs) represent a frequent medical condition[1–3] in which typical symptom presentation,[4,5] diagnostic criteria,[6] and multimodal treatment options[1,3,7] are well established, but only little is known about the underlying neuropathophysiological mechanisms causing the diverse symptoms.[8] Recent pathophysiological models focus on a multifactorial origin of FND in the framework of a stress-diathesis model[9,10] (from the ancient Greek term "diathesis" = predisposition) integrating predisposing, precipitating and preceding risk factors,[3,11,12] and evaluate state versus trait markers of the disorder.[13,14] Studying biopsychosocial vulnerability factors is thus of utmost importance and could further explain the development of FND symptoms in a subgroup of (biologically) vulnerable individuals with certain psychosocial risk factors.[11,15]

Negative life events have recurrently been reported in FND,[12,16–18] traditionally highlighting the role of sexual and physical abuse during childhood as preceding risk factor.[12,18,19] Moreover, severity and frequency of childhood abuse could be linked to symptom severity.[20] Similarly, symptom onset and severity could be connected to recent adverse social-occupational life events with a partial link to early childhood physical and sexual abuse,[19] highlighting the importance of type but also timing of trauma. In this regard, a recent meta-analysis confirmed an increased frequency of childhood and adult adverse life events and abuse in FND patients compared to healthy controls (HC) and psychiatric control patients.[21] Additionally, emotional neglect was identified to be much stronger associated with the symptom development, and thus weakened the dominating role of sexual abuse in the suspected aetiology of FND.[21]

Neuroimaging studies intensively investigated the relationship between traumatic life events, symptom presentation and brain functional- and structural abnormalities in FND. As such, structural alterations in limbic and motor regions could be associated to childhood abuse and symptom severity,[22–24] whose effect was even more pronounced in women.[25] Similarly, an aversive emotional-stimulus dependent alteration of cortico-limbic and limbic-motor brain networks, involving regions such as the hippocampus,[26–28] the amygdala,[28,29] the supplementary motor area (SMA[26]) and the prefrontal cortex (PFC[26,27]) have been identified in FND. Noteworthy, hippocampal deactivation is suggested to disinhibit the hypothalamus-pituitary-adrenal (HPA) axis, triggering a stress response,[30,31] resulting in the release of stress hormones such as cortisol.[32] HPA-axis alterations – as for example observed under chronic stress – have been associated with neuroanatomical changes, particularly in the hippocampus, the amygdala, or the PFC[33,34] which was attributed to a potential neurotoxic effect of glucocorticoids.[33,35]

In FND, some studies suggested that patients have prominent hyperarousal, as stress markers of the autonomic nervous system were found to be increased.[36–38] Only few studies, however, analysed cortisol in FND,[36,39–43] – as a measure of the adaptive (slow) stress response[32] – and the results were inconsistent. As such, decreased morning[41] and basal diurnal[43] cortisol were reported, in contrast to no differences[42] or increased basal diurnal cortisol compared to levels in HC.[39,40] This is explained essentially by methodological issues: studies were conducted using small sample sizes, focusing on only one particular symptom type, or within different test settings, potentially biasing the results.[44] This highlights the need to study the role of biological stress in relation to its neurological-, and psychological correlates, which could advance the understanding of pathophysiological mechanisms in FND and could generalize previous findings.

We set out to study alterations in the HPA-axis in a transdiagnostic approach across a large cohort of FND patients with mixed symptoms in a standardized domestic setting, to minimize biases of experimental setting. We adapted a transdiagnostic approach, as this efficiently targets the commonalities across the different symptom types. The primary aim was to assess the cortisol awakening response in FND compared to HC. The secondary aim was to evaluate the relationship between HPA-axis dysfunction, volumetric brain alterations and preceding trauma, and to discuss their potential role as predisposing (trait) versus precipitating factors.

Materials and methods

Participants

The study was conducted at the University Hospital Inselspital Bern, Switzerland. We included data of 86 FND patients with motor (F44.4) and sensory symptoms (F44.6), with functional seizures (F44.5), mixed symptom type (F44.7), and persistent postural-perceptual dizziness (PPPD). Board-certified neurologists confirmed the diagnosis of FND according to DSM-5[6] and using positive signs.[45] We included 76 age-and sex matched HC. Due to COVID-19 pandemic regulations in the hospital, no HC older than 65 years were allowed to be invited, and thus FND patients older than 65 years were not matched. Exclusion criteria were: 1) major neurological comorbidities, 2) a current severe psychiatric condition (acute suicidality, active psychotic symptoms), 3) alcohol or drug abuse, 4) pregnancy or breast-feeding, 5) contraindications for MRI and 6) insufficient language skills. The study was approved by the Competent Ethics Committee of the Canton Bern (SNCTP000002289) and conducted according to the Declaration of Helsinki. All subjects provided written informed consent.

Saliva samples

Saliva samples were collected according to the consensus guidelines of Stalder,[44] concerning design and strategies to control for adherence, and to account for covariates. All participants were instructed in an initial face-to-face appointment and received written take-home instructions and a self-reported diary. We assessed smoking habits, and for female participants information about their menstrual cycle and intake of hormonal contraceptives, as they represent potentially confounding factors of cortisol secretion.[44,46,47] Saliva was collected within a domestic setting, and a sampling date convenient for the participant was set. A reminder was sent by e-mail the evening prior to the sampling date. Participants were asked to collect nine saliva samples throughout the day by chewing for 2 minutes on a cotton swab (Salivette collection devices, Sarstedt, Rommelsdorf, Germany). Samples were taken directly upon awakening, 15-, 30- 45- and 60 minutes post awakening and further at 2-, 3-, 4- and 5 p.m. Participants were instructed to complete the five samples before breakfast and to refrain from heavy meals, fruits or fruit juices, coffee, carbonated soft drinks, chewing gum, smoking, teeth brushing or strenuous physical activities during the sampling in the morning and 45-60 minutes prior to sampling in the afternoon. Participants were instructed to note their wake-up time, any deviations from the

sampling time and potential confounds in their self-reported diary. Participants were free to wake-up naturally or using an alarm clock and to follow their daily routine as usual. Saliva samples were collected the next day, centrifuged (10 min at 3900 rpm and room temperature) and frozen at -20 °C.

Demographic, behavioural, and clinical characteristics

Symptom severity was evaluated using the Clinical Global Impression (CGI) score (zero = no symptoms to seven = among the most extremely ill patients) and the Simplified Version of the Functional Movement Disorder Rating Scale (S-FMDRS[48]). Duration of symptoms was calculated from onset of symptoms to date of the study inclusion (in months). Use of psychotropic medication (i.e., benzodiazepines, opioids, antidepressants, neuroleptics, and antiepileptics), as well as corticosteroid medication were recorded. Mood was assessed using the Spielberg State-Trait Anxiety Inventory (STAI[49]) and the Beck's Depression Inventory (BDI[50]). Sleep quality of the night prior to saliva sampling was assessed using item four and five of the Leeds Sleep Evaluation Questionnaire (LSEQ[51]).

Traumatic life events

Traumatic life experiences were measured using the Traumatic Experiences Checklist (TEC[52]). The TEC is a 29-item self-reported questionnaire which assesses the presence of diverse physical, emotional, and sexual traumata including age, relationship to the perpetrator, and the self-reported impact of the respective trauma. The TEC was scored using the syntax available at http://www.enijenhuis.nl/tec. Based on the syntax we computed 1) the overall number of experienced traumata (sum of all items), 2) six individual trauma severity subscores (determined by subjective impact and age of trauma for emotional neglect, emotional abuse, physical abuse, sexual harassment, sexual abuse, and bodily threat), and 3) developmental composite scores calculating experienced trauma according to the age ranges of 0 to 6 years, 7 to 12 years, 13 to 18 years and > 19 years. Additionally, we computed duration and relationship to the perpetrator for each trauma subscore. The duration of trauma was calculated using the maximum duration within those questions belonging to each trauma subscore. The relationship to the perpetrator was coded into categorical variables being one: inner-family circle (parents, siblings, partner), two: outer-family circle (relatives), three: friends and acquaintances, four: strangers. Additionally, to focus on trauma occurring only during childhood, we used the Childhood Trauma Questionnaire

(CTQ[53]); a 25-item self-reported questionnaire which assesses childhood trauma across five domains including emotional- and physical abuse and neglect, and sexual abuse.

Saliva samples analysis

Salivary cortisol was analysed by a commercial saliva-specific competitive enzyme immunoassay (cELISA, Salimetrics, Newmarket, United Kingdom). The manufacturer states a functional sensitivity of 0.28 ng/mL, and cross-reactivity for 14 endogenous and synthetic steroids is reported to be <1% each. The assay had been used according to the manufacturer's protocol. Intra- and inter-assay coefficients of variation were 4.5% and 4.8%, respectively.

Neuroimaging data acquisition and pre-processing

To investigate neuroanatomical differences between patients and controls, we used a voxel-based morphometry approach. Anatomical images were acquired for all subjects except of three FND patients and three HC. MRI sequence and pre-processing is detailed in the Supplementary Material.

Statistical analysis

Behavioural data

Statistical analyses were performed using *R* software (version 4.1.2.) and MATLAB (R2017b, MathWorks Inc., Natick, USA). Questionnaire data were tested for normality using Shapiro-Wilk's test. Normally distributed data were analysed using two-sample t-test, highly skewed data using Wilcoxon rank sum test. Questionnaires with subscores were corrected for multiple comparisons using false discovery rate (FDR). Categorical data were analysed using Chi-squared test (sex) and Fisher's exact test (menstrual cycle and relationship to perpetrator (TEC)). To determine significance, alpha-level was set at $P < 0.05$.

Biological data

We analysed two metrics to assess cortisol levels: the cortisol awakening response (CAR) and the diurnal baseline cortisol (DBC).

The CAR describes the rapid increase in cortisol secretion across the first 30 to 45 minutes upon awakening and thus, represents the dynamic changes of cortisol secretion occurring upon awakening.[44,54] It has been shown that the intraindividual stability is relatively high and subtle changes in HPA-axis function regarding environmental noise can be detected with high accuracy.[47] To assess group cortisol differences in the CAR, a repeated measures ANOVA was used on the fitted data of the five morning samples (wake-up until 60 min post-awakening) using a linear mixed model with fixed effects of factor group and timepoint, and using age, sex, smoking, wake-up time, BDI, STAI, hormonal contraception, corticosteroid medication, psychotropic medication, menstrual cycle, menopause, and sleep quality as covariates of no interest.[46]

The DBC represents the dynamic changes of cortisol throughout the afternoon (from 2 p.m. to 5 p.m.). To analyse the DBC, the same analysis was performed as in the CAR using the four samples in the afternoon. For the analyses of the CAR and the DBC, we excluded data from eight FND patients and nine HC as they did not properly adhere to the saliva sampling protocol with either missing samples (N = 3) and/or delays (N = 16) (strict sampling accuracy margin of $\Delta t > 5$ min for post-awakening samples and $\Delta t > 15$ min for afternoon samples[44]).

As we were interested in examining the multivariate pattern of correlation between cortisol and other variables (see below), single estimates of the CAR and the DBC were calculated using area-under-the-curve (AUC) based measures, as recommended in methodological consensus guidelines.[44,55] As such, the post-awakening cortisol concentration (PACC) and the diurnal baseline cortisol concentration (DBCC) were computed. The PACC describes the summed cortisol concentration across the first five samples in the morning. The DBCC represents the cumulated cortisol concentration of the four afternoon samples. As a measure for the PACC and DBCC, the AUC with respect to ground (AUC_G) was calculated. Additionally, as a (static) measure for the CAR, the AUC with respect to increase (AUC_I) was calculated on the five morning samples (CAR_i).[44] AUC-based measures were calculated according to Pruessner.[54] Three subjects were excluded for calculating the AUC-based measures due to missing samples. Subjects reporting delays were included, as the AUC formula can account for sampling delays (see Supplementary Material and Supplementary Fig. 1 for more details). All analyses were repeated in females only Supplementary Fig. 9.

Imaging data

To analyse between group differences of cortical volumes, we firstly applied a general linear model on the smoothed whole-brain anatomical images within SPM12. Second, given the *a priori* hypothesis of the hippocampus and the amygdala being particularly vulnerable to anatomical changes in the context of chronic stress,[33,34] we analysed volumetric differences in those two regions. As such, we performed two region-of-interest (ROI) analyses using the corresponding ROI masks, derived from the automatic anatomic labelling atlas 3 (AAL3[56]). Whole-brain, as well as ROI analyses were corrected for multiple comparisons using a family-wise error (FWE) rate at $P < 0.05$, and total intracranial volume (TIV), age, sex, depression, and anxiety were added to the analysis as covariates of no-interest. Lastly, we extracted subject-wise estimates of the mean ROI volumes for external analyses. All analyses were repeated in females only Supplementary Fig. 10, Supplementary Table 6.

Multivariate pattern of correlation

In a last step, we applied partial least squares correlation (PLSC[57,58]) to evaluate multivariate patterns of correlation between behavioural data (trauma scores), cortisol AUC_G and AUC_I measures (CAR_i, PACC, DBCC), and volumetric data (mean ROI volume) in FND patients and healthy controls. For the PLSC analysis, only those subjects were included of which salivary cortisol (FND = 84, HC = 75) and imaging data (FND = 83, HC = 73) were complete. Data was standardized and a correlation matrix was calculated between the two sets of variables. To find individual weights of the corresponding data tables (cortisol data, volumetric data, trauma scores), a single value decomposition (SVD) was applied on the correlation matrix. The SVD leads to different correlation components consisting of a set of design weights and outcome weights (saliences), indicating the strength of contribution of each weight to the multivariate pattern. The weights were used to calculate two sets of latent variables as such that the covariance was maximized. Significance was evaluated by permutation testing (5000 permutations). Stability of the weights was assessed using bootstrapping (200 bootstrapping samples). PLSC allows for examining the relationship between multiple variables with different attributes. We used the publicly available PLS toolbox for MATLAB (https://github.com/FND-ResearchGroup/myPLS_SL.git), the use of which has already been described in other studies.[59,60]

We conducted three individual PLSC analyses; First, we used the cortisol values as design variables, and TEC severity scores, developmental scores, duration of trauma, and relationship to the perpetrator as outcome variables to evaluate multivariate pattern of correlation of trauma history and HPA-axis dysfunction. Second, we used the volumetric data of the whole-brain, as well as hippocampus and the amygdala alone (normalized for TIVs) as design variables, and age, sex, and cortisol values as outcome variables to evaluate the multivariate pattern of correlation between cortisol and changes in brain volume. Lastly, we evaluated in patients only the relationship of the aforementioned factors with clinical data (i.e., symptom severity, and duration of symptoms), Supplementary Figures 6 – 8.

Data availability

The data are not publicly available due to restrictions demanded by the administering institution to guarantee the privacy of the participants. The data can be shared upon request.

Results

Clinical, behavioural, and demographic characteristics

Data from 86 FND patients and 76 age- and sex matched HC were included in this study. Demographic, behavioural, and clinical data are presented in Table 1. The most common symptom types were sensorimotor deficit (38.7%), gait disorder (21.5%), and/or tremor (14.6%). Level of diagnostic certainty for functional seizure patients were: seven probable, three clinically established, and four documented, according to diagnostic criteria of LaFrance.[61] Five patients were currently under corticosteroid medication, four of them only in a topical form (nasal spray) used irregularly on demand, and one patient was under oral prednisone medication. Patients using sprays resigned from using them on the day of saliva collection. FND patients and HC significantly differed in their smoking habits (more smokers in FND), their BDI, and STAI scores (more depression and anxiety in FND).

Trauma

Traumatic life events

(1) Overall number of experienced traumata (TEC): FND patients experienced significantly more total traumatic events compared to HC (reported as mean ± SD: FND 6.78 ± 4.37, HC 4.21 ± 4.22, $Z = 4541$, $P < 0.001$), Fig. 1A.

(2) Trauma severity scores (TEC): FND patients reported significantly more emotional neglect (FND 5.26 ± 6.32 vs. HC 2.4 ± 4.68, $Z = 4247$, $P = 0.002$), Fig. 1B.

(3) Developmental composite scores (TEC): FND patients reported significantly more traumata occurring in the age range from 0 to 6 (FND 3.43 ± 4.87 vs. HC 2.08 ± 3.93, $Z = 3810$, $P = 0.43$) from 7 to 12, (FND 4.71 ± 4.81 vs. HC 3.07 ± 4.17, $Z = 3962$, $P = 0.043$) and > 19 years old (FND 2.9 ± 4.03 vs. HC 1.26 ± 2.24, $Z = 3840$, $P = 0.01$), Fig. 1C.

(4) Duration of trauma (TEC): FND patients reported a longer duration of emotional neglect as compared to HC, i.e., 4.5 years longer (FND 6.95 ± 1.2 years vs. HC 2.36 ± 0.6 years, $Z = 3984$, $P = 0.01$), Fig. 1D**.** No significant differences were found with respect to duration of trauma for the other subscores.

(5) Relationship to the perpetrator (TEC): In FND patients, emotional neglect occurred more often through members of the inner-family circle (two-sided, $P = 0.006$). No significant differences were found in the other subscores.

Childhood trauma

FND patients reported significantly more childhood emotional abuse (CTQ scale reported as mean ± SD: FND 10.1 ± 5.1, HC 8.2 ± 4.2, $Z = 4028$, $P = 0.02$), emotional neglect (FND 11.1 ± 5.1, HC 8.8 ± 4.2, $Z = 4194$, $P = 0.009$), physical abuse (FND 7.3 ± 4.0, HC 5.9 ± 2.0, $Z = 3875$, $P = 0.03$), and physical neglect (FND 7.7 ± 3.1, HC 6.79 ± 2.83, $Z = 3935$, $P = 0.03$), Supplementary Fig. 2.

Salivary cortisol

A significant main effect of group was found for the CAR ($F(1,680) = 28.81$, $P < 0.0001$) with lower levels in FND than HC. Post-hoc multiple comparisons between group and timepoints, showed that FND patients and HC significantly different in their cortisol levels at timepoints 30’

upon awakening, and almost reached significance at timepoint 15'-, 45'-, and 60' upon awakening (P = 0.052), Fig. 2. No significant differences were found in the DBC.

Volumetric brain alterations in FND patients

On a whole-brain level, significant group differences were found between FND patients and HC in five clusters at thresholds of P_{FWE} = 0.05, Fig. 3A and Table 2. These clusters included the following regions with decreased volumes in FND compared to controls: Left superior temporal gyrus, left gyrus rectus, bilateral amygdala, hippocampal- and parahippocampal gyri, as well as dorsolateral prefrontal gyri.

In line with the results on a whole-brain level, we confirmed our *a priori* hypothesis of a reduced hippocampal- and amygdalar volume in FND patients using an inclusive brain mask at thresholds of P_{FWE} = 0.05, Fig. 3B, Supplementary Table 1,2. Upon extraction of ROI volumes for external analyses, we found that the hippocampus, as well as amygdala volume were significantly smaller in FND patients compared to HC ($F(1,614) = 102$, $P < 0.001$). Post-hoc Tukey's HSD test revealed a significant difference between FND patients and HC in 1) the left hippocampus ($P < 0.001$), 2) the right hippocampus ($P < 0.001$) (Fig. 3A, upper panel), 3) the left amygdala ($P = 0.016$), and 4) the right amygdala ($P = 0.025$) (Fig. 3B, lower panel).

Relationship between trauma and cortisol

To evaluate relevance of experienced trauma on the single estimates of the cortisol measures (CAR_i, PACC and DBCC) in FND patients and HC, we first conducted a behavioural PLSC including TEC severity scores, developmental scores, duration of trauma, and relationship to the perpetrator as outcome variables. One PLSC component was found to be statistically significant based on the permutation testing (P = 0.033). The outcome and cortisol saliences of the previously mentioned component are shown in Fig. 4. Yellow highlighted weights indicate that they were found to be robust (with the green dots representing the cortisol salience weights) and can be interpreted similarly to correlation coefficients as the data was standardized. Based on the PLSC results, a significant positive correlation was found in patients between the morning cortisol values (CARi, PACC) and the relationship to the perpetrator of physical abuse – meaning that the more familiar (inner-family circle) the perpetrator was, the higher the cortisol values. A significant negative correlation was found in patients between the morning cortisol values (CARi, PACC) and 1) the duration, and 2) severity of emotional neglect – meaning that the longer and

more severe the emotional neglect, the lower the cortisol values. In HC, a positive correlation was found between cortisol values and 1) trauma occurring during late adolescence and 2) adulthood – meaning that the more trauma happened during late adolescence and adulthood, the higher the cortisol levels.

Relationship between cortisol and brain volume

To examine the potential relationship between single estimates of the cortisol measures (CAR_i, PACC and DBCC) and changes in whole-brain, respectively hippocampal- and amygdalar volumes in FND patients and HC, we conducted a PLSC including cortisol values as outcome variables and imaging data as design variables. No significant PLSC components were found when using the mean cluster volumes from the whole-brain analysis as design variables. When using the results from our ROI analysis (i.e., hippocampal and amygdalar volume), one PLSC component was found to be statistically significant (permutation testing, $P = 0.021$). The outcome and imaging saliences are shown in Fig. 5.

Based on this PLSC analysis, a significant negative correlation was found only in HC between the brain volumes of the bilateral hippocampus and the bilateral amygdala and 1) the age – meaning that the older the subject, the smaller the brain volume – and 2) CAR_i – meaning the smaller the brain volume, the higher the cortisol levels. No multivariate pattern of correlation between brain volumes and cortisol data was found in FND patients.

Relationship with symptom severity in FND

No significant multivariate correlation was identified in patients, when using symptom severity as outcome variable, and trauma scores, single estimates of cortisol measures, or brain volumes, independently, as design variables, Supplementary Fig. 6-8.

Discussion

Our findings provide biopsychological evidence for the stress-diathesis model in FND (state versus trait). We identified a reduced cortisol awakening response in a transdiagnostic approach in FND patients. Moreover, we linked the potential HPA-axis dysregulation to prolonged preceding emotional neglect, pointing towards a long-term process resulting in a maladaptive HPA-axis sensitization. Lastly, we identified anatomical changes in the superior frontal gyrus, the superior temporal gyrus, the hippocampus, and the amygdala. In FND, however, reduced cortical

volumes were not associated with cortisol – what would have pointed towards a potential neurotoxic effect, nor with symptom severity – what could have explained a state related change. These findings put in question whether the here found results represent a direct state effect of FND, a biological trait factor, or a combination of both as will be further discussed below. A schematic representation of the here discussed results are displayed in Fig. 6.

Only few studies investigated cortisol levels and the stress response in FND patients. Consistent with our results, Chung[41] detected a blunted CAR in 32 children with FND (mixed symptoms) assessed using two saliva samples in the morning (at wake-up and 30 min later), which were partially collected in a domestic setting. Likewise, a study in 15 female functional seizure patients identified lower serum cortisol levels in the morning as compared to HC with a history of abuse.[43] Contradictorily, a study in which 33 motor FND patients and 33 HC were hospitalized overnight, no difference in morning cortisol levels were found.[42] This discordance might be explained by the testing conditions: a non-familiar environment (e.g., hospitalization[42]) might introduce alterations in cortisol levels that covary with psychosocial factors and might not represent the clinical status of patients.[44,62] Consistent with our results, no group differences in the basal diurnal cortisol levels were found in 19 functional seizure patients,[36] nor in motor FND patients (N = 16[39], N = 33[42]). Contrarily, a group effect with higher basal diurnal cortisol levels in the afternoon was found in motor FND,[39] mainly driven by stress, as well as in functional seizure patients,[40] mainly driven by experienced sexual abuse. Lastly, cortisol secretion was studied in response to stress. Using the Trier Social Stress Test, two studies reported a comparable stress response in FND patients as to HC indicating a normal adaptation to social stress situations.[36,39] In summary, previous results on cortisol in FND show a large heterogeneity, mainly explained by methodological issues: each of the studies was conducted in a different setting (stress test[36,39,40] versus no stress test and domestic setting versus hospitalized[41–43]), assessing different measures of cortisol (i.e., morning versus basal versus stress response), which in most cases prevents a direct comparison between results. Our transdiagnostic approach has the advantage of having a large sample with mixed symptoms, which ensures a better generalizability in comparison to previous studies focused on small subgroups of FND patients.

Additionally – and firstly in FND, we identified an inverse relationship between cortisol measures and various dimensions of emotional neglect (assessed using the TEC), whereas no association with symptom severity or duration of symptoms was detected. As such, a significant

multivariate pattern of correlation was found in patients but not in controls, between lower morning cortisol levels and higher duration and severity scores of emotional neglect (measured by the TEC). Specifically for emotional neglect, exposure was in average 4.5 years longer in FND as compared to HC. In general, adverse experiences occurred more frequent in early childhood in FND than in HC, even though this effect was not specific to emotional neglect but was found across all traumatic experiences. This result is consistent with the findings on the CTQ, in which increased neglect and abuse was found in FND across all trauma subscores except for sexual abuse. Particularly, the role of neglect as predisposing factor of FND has been highlighted by the results of a meta-analysis of 34 case-control studies including 1405 patients showing odd ratios (OR) of 5.6 for FND patients compared to control populations, which was higher than for sexual and physical abuse (OR 3.3 and 3.9 respectively).[21] Our results go further than confirming an association between emotional neglect and FND in demonstrating that both the severity and duration of emotional neglect are more pronounced in FND. The effect of maltreatment on different expressions of psychopathology has been shown to depend on the developmental period, severity, and frequency of trauma exposure.[63,64] In FND, no clear consensus on the role of trauma type, timing and number of traumatic events is known, with the exception that early-onset FND was rather associated to childhood sexual abuse[65] when late-onset was associated to physical trauma.[66] In sum, our results add to previous knowledge that trauma predisposes to FND, highlighting the importance of emotional neglect. Additionally, we first showed that in FND exposure to early and long-lasting emotional neglect might contribute to disrupting the biological regulation of stress, as reflected by the association with blunted CAR. This is further supported by the absence of an association between CAR and symptom severity, as an association between CAR and symptom severity would rather indicate a (subacute) disease-related ('state') change of the HPA-axis.

Thereby, dysregulation of morning cortisol secretion might represent a downregulation of the HPA-axis following initial high levels of cortisol in response to long-term stress.[67] A proposed mechanism of action is the suppression of the negative feedback inhibition of cortisol.[33,34] Under normal health conditions, an acute stressor would activate the HPA-axis and subsequent cortisol secretion through the amygdala. The amygdala is strongly regulated by the PFC and the hippocampus, which are responsible for the integration of information on threat stimuli. When the stressor is removed, a negative feedback inhibition is induced through the hippocampus and the HPA-axis itself, reducing the cortisol secretion. In a chronic state of hypervigilance to

stressors, the HPA-axis is tonically inhibited through the hippocampus, as a result of suppressed negative feedback inhibition due to HPA-axis sensitization (maladaptive habituation) to the stressor. Correspondingly, an overreactive HPA-axis has been observed in early phases of chronic stress, whereas a downregulation corresponds to subsequent, sustained phases of chronic stress.[68] Hence, the prolonged exposure to emotional neglect in FND patients might reflect a long-term process resulting in the downregulation of the HPA-axis, as represented in the flattened CAR. At the same time, it is suspected that glucocorticoid receptors become more sensitive to enhanced cortisol levels during early phases of chronic stress, and consequently to the increased neurotoxic effects of cortisol.[69–71] Chronic stress indeed has been repeatedly associated with neuroanatomic alterations in regions expressing a high glucocorticoid receptors density i.e., hippocampus, PFC, and amygdala (for review[33,34]). In FND, a volume reduction of the hippocampus has previously been found to inversely relate to trauma history.[25] No data on cortisol was available in this study but it was hypothesized that the hippocampal atrophy might be mediated by changes in stress biomarkers such as cortisol. However, large variation in hippocampal volumes has also been described in healthy populations, irrespective of chronic stress or trauma history, suggesting that reduced hippocampal volume may represent a trait factor rather than a disease-related feature (state).[72] In line with these findings, our results on smaller hippocampal and amygdalar volumes compared to HC, and the absence of a correlation with cortisol measures nor with symptom severity suggest that these anatomical variations rather represent a trait factor for FND, in terms of a biological predisposition. Interestingly, while some studies neither identified a relationship between cortical volumes and symptom severity,[23,73,74] recent studies inversely correlated symptom severity to lower volumes in regions other than the hippocampus, such as the left insula,[22,25,75] precentral gyrus,[75] as well as the temporo-parietal junction.[76] Therefore, regional differences in cortical volume might be linked to trait-vulnerability (e.g., hippocampus) while others might be linked to disorder-related pathophysiological changes (state). However, additional research is needed to disentangle the role of regional structural abnormalities in the pathophysiology of FND. On the contrary in HC, the inverse relationship between subcortical volume and cortisol measures may represent a plasticity phenomenon in response to recent stress. In summary, a disease model including HPA-axis sensitization might contribute to the development of FND in terms of maladapting to long-term emotional neglect. Moreover, the here found reduced hippocampal and amygdalar volumes in

FND point towards a 'trait' biomarker for FND, which potentially decreases the resilience to stress.

Psychosocial stressors, HPA-axis sensitization and biological predisposition might represent transdiagnostic risk factors[77] which conjointly contribute to general psychopathology and symptom overlaps in neuropsychiatric disorders.[78] However, by way of example, about 15% of childhood maltreatment survivors do not develop mental health problems,[79] and further variations in psychopathology have been explained by individual resilience to stress.[78] Similarly, FND represents a disorder of multifactorial origin.[3] Biopsychological risk factors might interplay with other, yet unknown factors which might explain why a subgroup of vulnerable individuals develop FND and not any other psychopathology. Recently, research on resilience focuses not only on the exploration of eco-phenotypes (i.e., environmental factors), but also genetics and their interplay (endo-phenotypes, i.e., gene × environment interactions). As such, early life adversities may influence brain development and mental health outcome by means of (epi-) genetic mechanisms. The first two years of development is the critical window for emotional development and has been associated with increased risk for mental disorders and negative impact on the brain structure and function.[80,81] Emotional neglect during early childhood is often accompanied by social disentanglement and rejection, which prevents children to learn how to properly process emotions,[82–84] as found in FND populations.[26,85–87] In terms of gene × environment interactions, a genetic variation in the oxytocin receptor (*OXTR*) in subjects with a history of childhood emotional neglect was associated with reduced amygdalar and hippocampal brain volumes.[88] The role of oxytocin in emotion processing has been studied in infants (5-7 months old): infants with increased *OXTR* methylation rates showed enhanced response to aversive faces in a functional neuroimaging paradigm.[89] Epigenetic changes in the oxytocin pathway are as well of particular interest in FND, as increased *OXTR* methylation was demonstrated in a cohort of 16 FND patients compared to 15 HC.[90] Other genetic/epigenetic changes in FND have been very recently studied: Diez[28] linked history of childhood physical abuse to cortico-limbic brain network dysfunction in regions which *in situ* showed an overlap with high expression of genes involved in neuronal morphogenesis. Those findings firstly linked childhood trauma and its potential effects on brain function to a trauma-related functional brain reorganization in the context of a gene × environment interaction in FND. In the same line of research, tryptophan-hydroxylase 2 (*THP2*) polymorphism was associated with childhood trauma, symptom onset and severity, as well as amygdalar functional connectivity in FND.[91] In

summary, individual resilience factors might explain how early childhood emotional neglect potentially induce (epigenetically mediated) neurodevelopmental delays in individuals who later develop FND affecting brain structure and function of regions involved in emotion regulation which is reflected in a dysfunctional HPA-axis. Further research must be conducted to identify risk factors specific for FND.

Our study has several limitations. First, the measure of cortisol awakening response relies on self-reported diaries and deviations from the protocol cannot be fully controlled. To verify accurate execution of cortisol sampling, objective verification of awakening and sampling times are required,[92] e.g., using objective electronic monitoring systems, such as polysomnography or wrist actigraphy.[93] We did not use such objective tools but minimized the risk of error of self-report data by thoroughly instructing our participants, agreeing on an appropriate day for the sampling, and explaining them the importance of properly adhering to the protocol and/or reporting deviations from the protocol. Second, we collected saliva samples on only one day, thus cortisol alterations might represent fluctuations due to situational aspects rather than a long-term trait.[44] Thirdly, salivary cortisol only indirectly measures HPA-axis activity, as it depends on levels of other biological factors such as corticotropin releasing factor, adrenocorticotropic hormone, or estrogens.[94] Nonetheless, salivary cortisol is considered to be a good measure of allostatic load, and a useful biomarker in stress research.[47,94] Another limitation in studying the role of trauma lies in methodological issues as self-report questionnaires can have recall bias.[21] Detailed interview technique,[95] are less prone to recall bias but are time-consuming and requires appropriate training of study personnel, which limits its feasibility in larger cohorts of participants. Lastly, our patient cohort has only been compared to HC, which prevents making conclusions on the specificity of the findings to FND in comparison to other stress-related disorders. We, however, corrected for depression and anxiety and excluded severe psychiatric conditions, therefore, we do not expect that the results are biased due to mood disorder comorbidities. The lack of systematic psychiatric evaluation – such as the psychiatric interview (SCID) – does not allow to check if the data could be confounded by a psychiatric co-morbidity (e.g., post-traumatic stress disorder), which is common in FND.[2,25]

Conclusion

Our findings point towards a multifactorial stress-diathesis model for FND. A flattened CAR might represent a long-term process in direct relation to severity and duration of emotional

neglect (state). Reduced subcortical volumes in FND did not relate to HPA-axis dysfunction and rather delineate a predisposing biological vulnerability, than a disease-related feature, thus potentially representing a trait marker for FND. In line with a stress-diathesis model, phenotypical variations in clinical presentation of symptoms must potentially be attributed to different contributions of a variety of diverse eco-phenotypes (e.g., trauma history) and endo-phenotypes (e.g., biological predisposition or trait markers). However, a causal relationship between HPA-axis dysfunction, trauma, and brain functional- and structural stress adaptation remains to be discovered. Longitudinal data would need to be assessed including the collection of behavioural, neuroendocrine, genetic, and neuroimaging data already in early childhood.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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Figure legends

Figure 1 Traumatic Life Events. (**A**) For visualization purposes, means and confidence intervals of overall number of experienced traumata (ranging from 0 to 29). (B) Means and confidence intervals of six trauma severity scores (determined by subjective impact and age of trauma, ranging from 0 to 13 for emotional neglect, emotional abuse, physical abuse, sexual harassment, and sexual abuse or from 0 to 24 for bodily threat). (C) Means and confidence intervals of developmental composite scores (across trauma subscores). (D) Means and confidence intervals of duration of trauma. Significance codes: $P^{***} < 0.001$, $P^{**} < 0.01$, $P^{*} < 0.05$. Results are FDR-corrected.

Figure 2 Cortisol Profile of FND patients and healthy. Mean and confidence intervals of daytime cortisol profile in FND patients and HC. Significance code: $P^{*} < 0.05$.

Figure 3 Results of voxel-based morphometry analysis. (A) Differential effect of voxel-wise comparison (HC > FND) with smaller grey-matter volume in FND in the hippocampus, parahippocampal gyri, amygdala, and dorsolateral frontal gyri. (**B**) Differential effect of mean ROI volume using a hippocampal mask (upper panel) and amygdala mask (lower panel) with smaller grey matter volume in FND. For both analyses, total intracranial volume (TIV), age, sex, depression (BDI), and anxiety (STAI) were added as covariates, thresholded on whole-brain level at $P_{FWE} < 0.05$. Significance codes: $P^{***} < 0.001$, $P^{**} < 0.01$, $P^{*} < 0.05$. A model corrected only

for TIV, age, and sex can be found in the Supplementary Material, Supplementary Fig. 3, Supplementary Table 3.

Figure 4 Partial least squares correlation (PLSC) results of the different cortisol measures (CARi, PACC, DBCC) in FND patients and healthy controls. The outcome (**A**) and cortisol saliences (**B**) of the significant PLSC component ($P = 0.033$) are presented. 5^{th} to 95^{th} percentiles of bootstrapping are indicated in the error bars and yellow highlighted bars indicate robustness. The height of the bar corresponds to the salience weight to the multivariate correlation pattern and can be interpreted similarly to correlation coefficients as the data was standardized. The permutation null distribution and the bootstrap mean percentiles are reported in Supplementary Fig. 4, Supplementary Table 4. Abbreviations: EN = Emotional neglect; EA = Emotional abuse; PA = Physical abuse; SH = Sexual harassment; SA = Sexual abuse; BT = Bodily threat.

Figure 5 Partial least squares correlation (PLSC) results of the imaging data (hippocampal and amygdalar volumes) in FND patients and healthy controls. The outcome (**A**) and imaging saliences (**B**) of the significant PLSC component ($P = 0.021$) are presented. 5^{th} to 95^{th} percentiles of bootstrapping are indicated in the error bars and yellow highlighted bars indicate robustness. The height of the bar corresponds to the salience weight to the multivariate correlation pattern and can be interpreted similarly to correlation coefficients as the data was standardized. The permutation null distribution and the bootstrap mean percentiles are reported in Supplementary Fig. 5, Supplementary Table 5.

Figure 6 The stress-diathesis model in functional neurological disorders. The aetiology of FND is multifactorial and depends on predisposing, precipitating, and perpetuating risk factors. Long-term exposure to stress can exert neurotoxic effects on regions particularly sensitive to cortisol. Moreover, it can alter the HPA-axis in terms of a maladaptive habituation. Distinct predisposing factors, i.e., 'trait' markers might influence the individual resilience to stress and the later development of psychopathology. Abbreviations: CRF = corticotropin-releasing factor, ACTH = Adrenocorticotropic hormone.

Table 1 Demographic, behavioural, and clinical data

	FND (N = 86)	HC (N = 76)	Statistics
Age, mean (SD), years, [range]	37.7 (14.2), [17–77]	33.1 (10.9), [18–62]	ns
Sex (females/males)	64/22	55/21	ns
Hormonal Contraception (yes/no)	27/37	18/37	ns
Menopause (yes/no)	14/50	10/45	ns
Menstrual Cycle[a]	15 anovulation 10 follicular 22 luteal 2 menstruation 7 ovulation	10 anovulation 3 follicular 33 luteal 1 menstruation 3 ovulation	Two-tailed $P = 0.05$*
Smoker (yes/no)	33/53	8/68	$X^2(1) = 15.2$, $P < 0.0009$***
Disease severity (CGI, median, quantile)	2 [1–4]	NA	
Disease severity (S-FMDRS, median, quantile)	6 [2 -12.75]	NA	
Duration of illness (in months)	75 (166)		
Symptom type[b]	45 sensorimotor 25 gait disorder 17 tremor 12 myoclonus 14 seizures 8 dystonia 7 PPPD 5 speech disorder 2 functional deafness 1 functional vision loss	*NA*	
ICD-10 Classification[c]	63 F44.4 7 F44.5 30 F44.6 8 F44.7 6 PPPD	*NA*	
Psychotropic medication	14 benzodiazepines 29 antidepressants 6 neuroleptics 9 antiepileptics 6 opioids	0/76	
Corticosteroids (yes/no)	5/81	0/76	
BDI score, mean (SD)	14.4 (9.96)	4.59 (6.28)	$Z = -7.61$, $P < 0.0001$***
STAI-S score, mean (SD)	37.2 (10.9)	32.1 (7.67)	$t(156.68) = 3.22$, $P = 0.002$**
LSEQ, mean (SD)	0.422 (0.169)	0.455 (0.15)	*ns*

[a]Menstrual cycle was indeterminable in 8 patients and 5 healthy controls (natural irregularity or continuous intake of hormonal contraception).
[b]Patients can present with several symptom types.
[c]Diagnosis of mixed FND (F44.7) was given when F44.4, F44.5, and F44.6 was present.
P*** < 0.001, P** < 0.01, P* < 0.05.

Table 2 Whole-brain voxel-based morphometric results with total intracranial volume (TIV), age, sex, depression (bdi), and anxiety (stai) as covariates of no interest

Cluster-level			Peak-level			Peak coordinates in MNI Space {mm}			Cerebral regions
P_{FWE}	P_{FDR}	Cluster extent	P_{FWE}	P_{FDR}	Peak voxel Z-score	*x*	y	z	
0.001	0.084	255	0.002	0.506	5.248	− 54	− 27	14	Left superior temporal gyrus
0.000	0.004	633	0.004	0.506	5.122	− 15	3	− 24	Left parahippocampal
			0.006	0.667	4.996	− 23	− 1.5	− 18	Left amygdala
			0.017	0.875	4.783	− 29	− 17	− 14	Left hippocampus
0.006	0.553	82	0.004	0.506	5.117	0	62	− 26	Left gyrus rectus
0.008	0.553	69	0.014	0.875	4.831	15	3	− 24	Right parahippocampal
			0.035	0.917	4.607	17	− 6	− 15	Right amygdala
0.009	0.553	61	0.019	0.875	4.753	− 11	59	− 15	Left superior frontal gyrus
			0.026	0.897	4.680	− 6	59	− 7.5	Left dorsolateral prefrontal gyrus

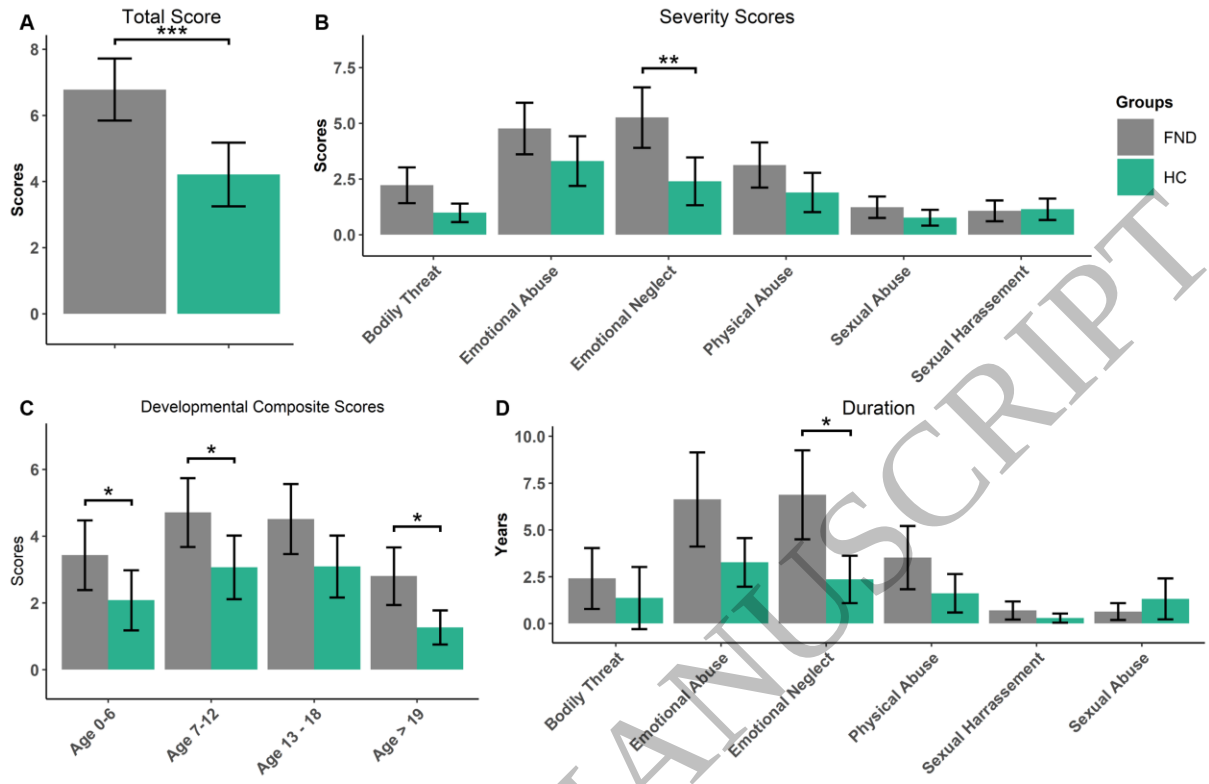

Figure 1
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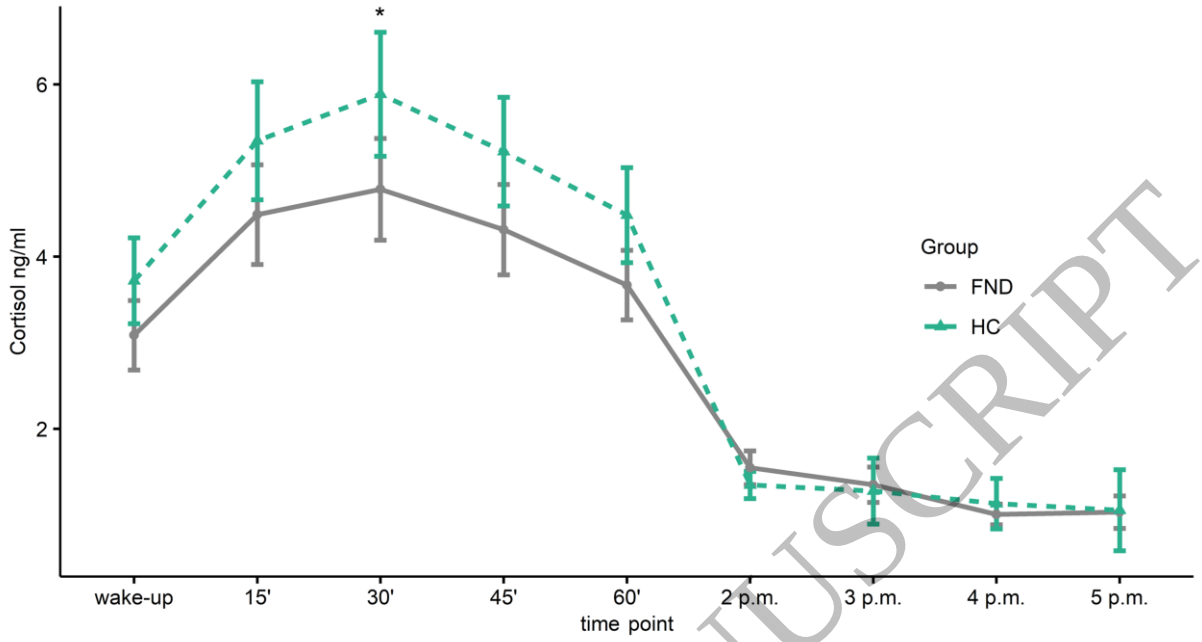

Figure 2
160x88 mm (.16 x DPI)

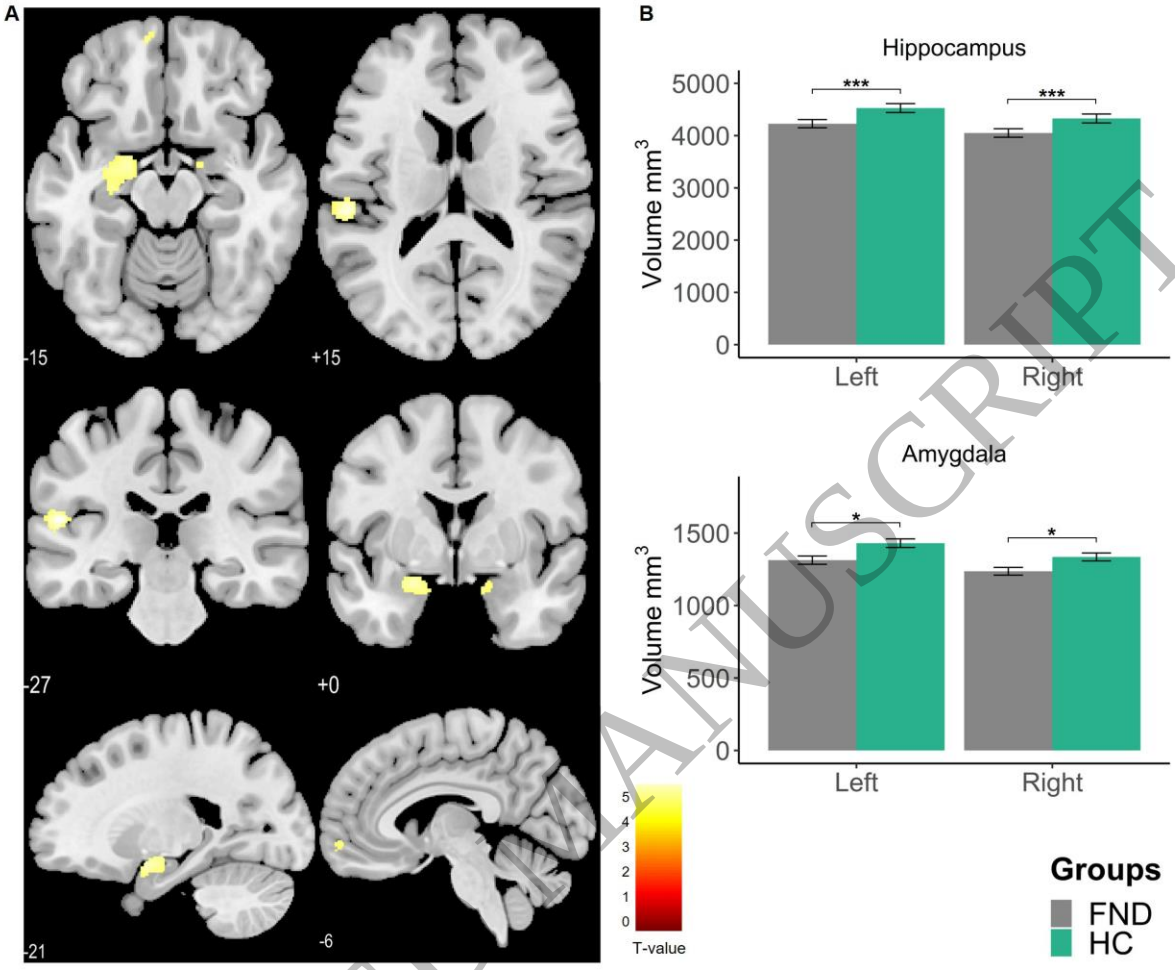

Figure 3
160x133 mm (.16 x DPI)

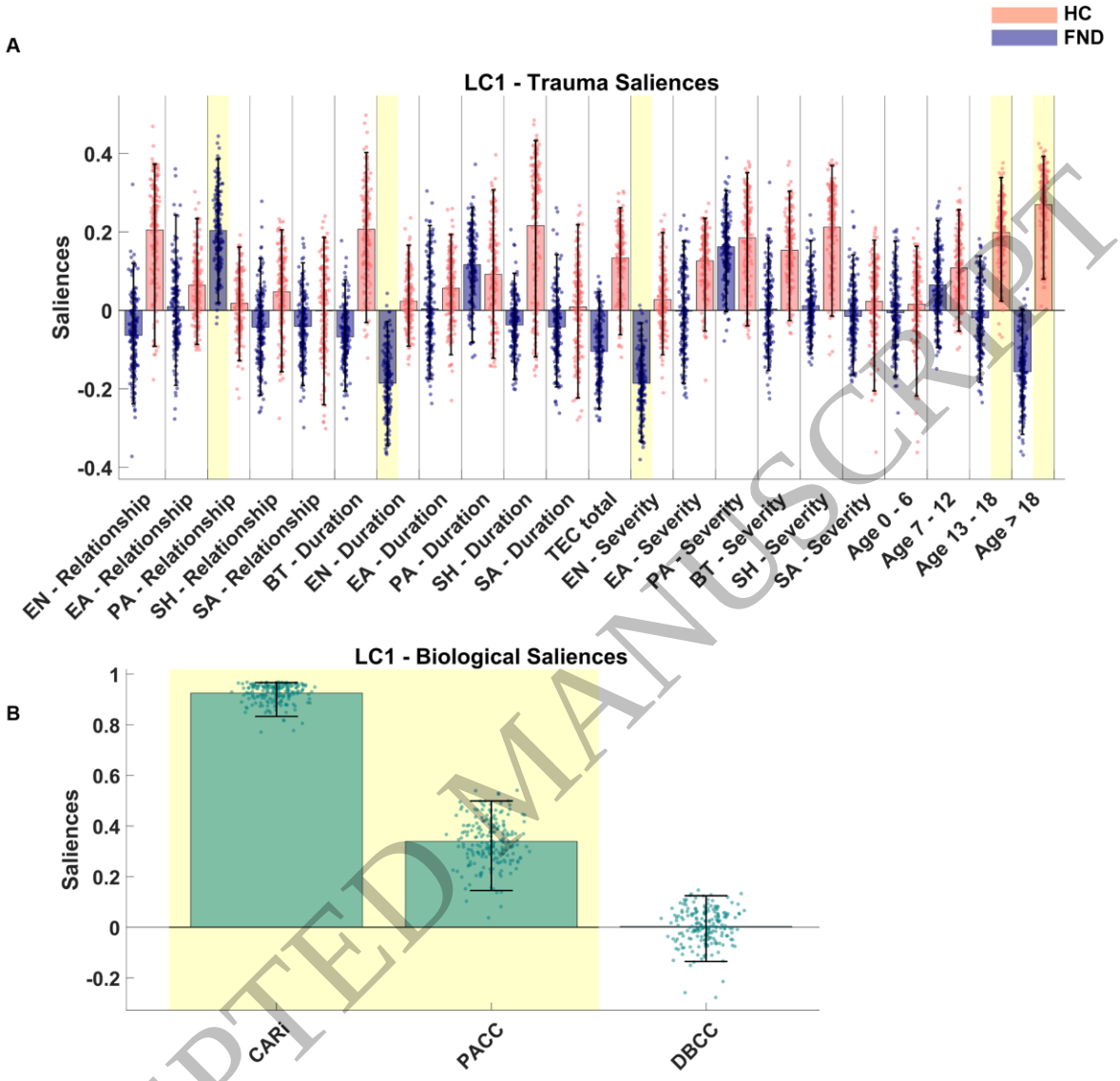

Figure 4
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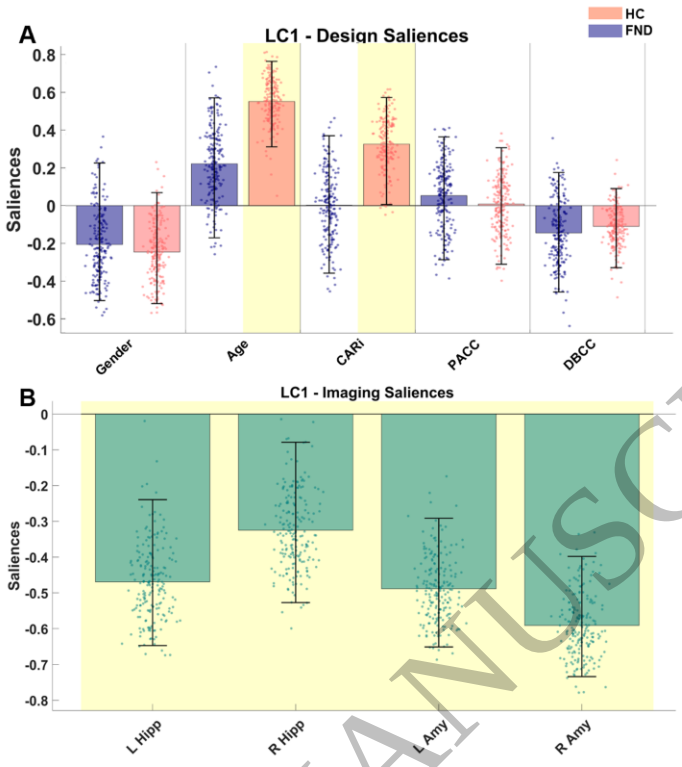

Figure 5
90x102 mm (.16 x DPI)

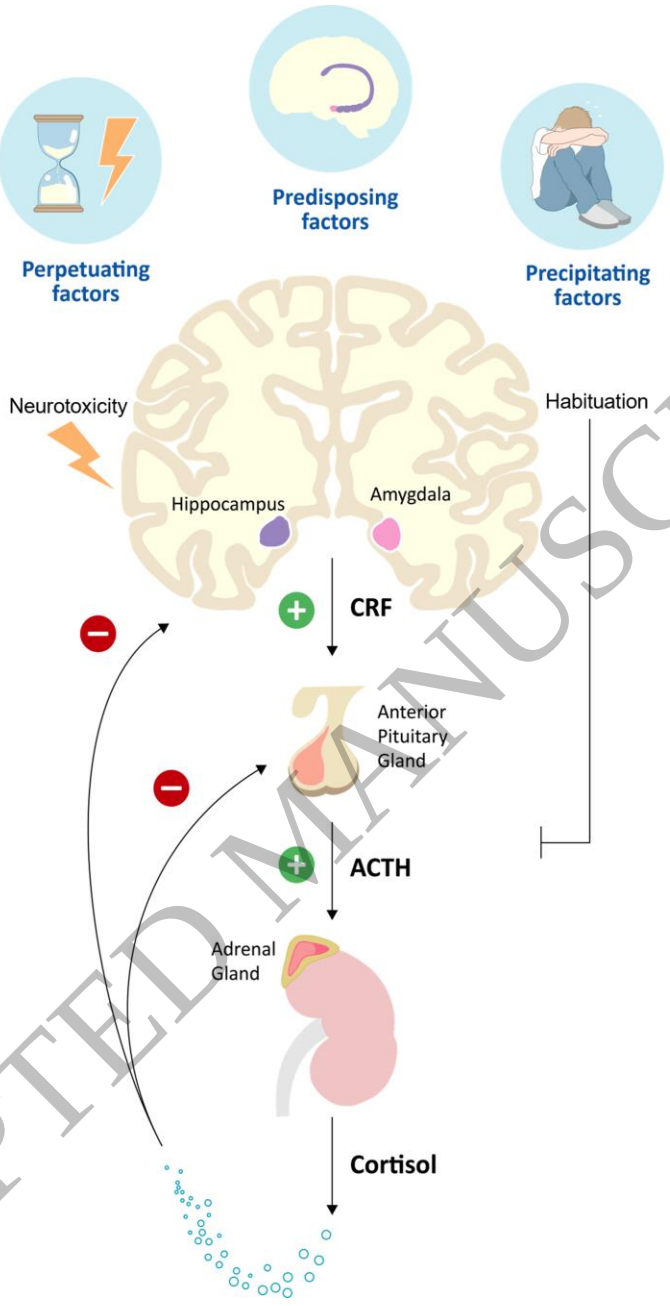

Figure 6
90x171 mm (.16 x DPI)