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# Cortisol in functional neurological disorders: State, trait and prognostic biomarkers

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ABSTRACT

*Objective:* Biological stress dysregulation, such as a flattened cortisol awakening response (CAR), has been identified in functional neurological disorder (FND). This longitudinal study aimed to explore whether CAR alterations in FND serve as *state* or *trait* biomarkers, assessing temporal changes in cortisol and clinical outcomes to test its *prognostic* value.

*Methods:* Salivary cortisol was measured in 53 patients with mixed FND at two visits separated by eight months (M0 and M8). CAR was calculated based on five consecutive samples, each taken with 15-min time intervals, collected upon awakening, whereas cortisol amplitude (CAmp) was calculated as the difference between the morning peak and the afternoon trough. Clinical outcome was assessed with the Functional Movement Disorder Rating Scale (S-FMDRS), Clinical global impression (CGI) scores for severity (CGI-S) and improvement (CGI-I) and the short-form health survey (SF-36).

*Results:* No differences in CAR levels were found between M0 and M8 regardless of clinical outcome (remained flattened). However, a good clinical outcome was associated with an earlier peak in the CAR ($p = .013$, odds ratio: 1.78; 95%-confidence interval: 0.095–1.13). A lower CAmp at M0 predicted a better outcome at M8 ($\beta = 1.14$, 95%-confidence interval:0.15–2.13, $p = .03$).

*Conclusion:* A flattened CAR might represent a *trait* marker for FND, when an earlier peak in the CAR may serve as a *state* biomarker. The CAmp demonstrates predictive power for clinical outcome, potentially representing a *prognostic* biomarker for FND. Further replication and follow-up studies are essential to confirm this suggested role of cortisol as a multifaceted biomarker of FND.

## 1. Introduction

Patients with functional neurological disorders (FND) exhibit neurological symptoms [1,2] for suggesting a multifactorial patho-physiological model involving stress and biological vulnerability [3–5]. Even though the diagnosis of FND is a rule-in diagnosis [6], and not only a diagnosis of exclusion [2], additional biomarkers are needed [7]. One possibility to investigate on potential biomarkers in FND is given by the hypothalamic-pituitary-adrenal (HPA) axis which is one of the main regulatory systems for the circadian cortisol cycle [8] and has been extensively studied regarding its regulatory function in the stress response [9–11]. A meta-analysis across eleven FND studies measuring baseline cortisol reported similar levels compared to healthy controls and between FND subtypes [12]. However, recent studies suffer from small sample sizes, poor control cohort quality, and measurement approach issues, impacting replicability.

An important attribute of proper HPA-axis function is the cortisol awakening response (CAR) defined by a rapid increase in cortisol secretion within 60 min after awakening [13,14], which is suggested to be a good measure to accurately detect fluctuations in HPA-axis activity [15]. Previously, we identified a blunted CAR in patients with FND as compared to healthy controls and could further link it to a history of severe and prolonged emotional neglect [3]. We proposed the flattened CAR to represent a *state* marker for FND suggesting a maladaptive neuroendocrine response to long-term psycho-social stress [16]. An unanswered question is whether this blunted CAR finding is linked to the

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presence of FND reflecting a stress dysregulation due to suffering from neurological symptoms and thus, can be considered a *state* marker of FND or if it is independent of the symptoms of FND and can be considered a *trait* marker [11,17,18].

A way to address this question is to conduct a longitudinal study and examine the change over time of both symptoms and the CAR. In addition, a longitudinal design allows for the identification of prognostic biomarkers, indicating biological factors at baseline/diagnosis predicting clinical outcomes at follow-up. In line with this, the CAR prior to treatment has been found to predict clinical outcome and symptoms severity in post-traumatic stress disorder (PTSD [19,20]), major depressive disorder (MDD [21]) or first episode psychosis [22], which however, is not yet reliable nor informative enough at the individual level to be considered a prognostic factors for any of these conditions in clinical settings.

In this study, we first set out to examine if cortisol represents a state or a trait marker for FND by investigating cortisol in a longitudinal design. As a secondary aim, we investigate prognostic abilities of cortisol regarding clinical outcome in FND.

## 2. Methods & materials

### 2.1. Participants and procedure

The study was conducted at the University Hospital Inselspital Bern, Switzerland. We included data of 53 FND patients with motor (ICD-10 code F44.4) and sensory symptoms (F44.6), with functional seizures (F44.5), mixed symptom type (F44.7), and persistent postural-perceptual dizziness (PPPD, ICD-11 code AB32) who completed an 8-month follow-up from a previously published cohort of 86 patients [3]. The FND diagnosis was established by a certified neurologist according to DSM-5 [23] and ICD-10 criteria [24]. Exclusion criteria were: 1) major neurological comorbidities, 2) a current severe psychiatric condition, 3) alcohol or drug abuse, 4) pregnancy or breast-feeding, and 5) inadequate proficiency in the Swiss national languages to understand the study protocol and provide informed consent. As the rate of dropout was high (53/86, see Supplementary Fig. 1 for reasons), we checked for a selection bias by comparing baseline clinical and behavioral scores at inclusion between the dropouts and those subjects who participated in the follow-up measurement (Supplementary Table 1). The study was approved by the local Ethics Committee (SNCTP000002289) and conducted according to the Declaration of Helsinki. All subjects provided written informed consent. The study procedure is described in [3] and detailed in the Supplementary Material.

### 2.2. Clinical outcome

Clinical status was assessed at M0 and M8 with two examiner-rated measures: the Simplified Version of the Functional Movement Disorder Rating Scale (S-FMDRS [25]) and the Clinical Global Impression Score for symptom severity (CGI-S, ranging from zero = no symptoms, to seven = among the most extremely ill patients). Additionally, the Clinical Global Improvement Score was assessed at follow-up (M8) to measure clinical improvement (CGI-I, ranging from 1 = very much improved, to 4 = no change, to 7 = very much worse). Furthermore, we used a patient-rated measure with the SF-36 [26] *general health* subscale (5 items), which refers to overall perception of well-being as reported by patients and thus integrates physical and mental health (see Supplementary Materials).

To investigate on longitudinal changes in cortisol with respect to clinical outcome (see part three and four of statistical analyses), patients were stratified into two groups concerning their clinical outcome at M8 with respect to M0 (clinical improvement vs. aggravation/unchanged) based on $\Delta_{S\text{-}FMDRS}$, $\Delta_{CGI\text{-}S}$ and CGI-I. A patient was deemed as "objectively clinically improved" if at least one of the three scores ($\Delta_{S\text{-}FMDRS}$, $\Delta_{CGI\text{-}S}$ and CGI-I) indicated improvement, with a difference of 1 point between M0 and M8 considered as a change in symptom severity. Second, a patient was labelled as "subjectively clinically improved" based on the minimal clinical important difference (MCID) of $\Delta_{SF\text{-}36}$ of 5.3 [27].

### 2.3. Statistical analysis

Statistical analyses were performed using *R* software (version 4.1.2.). To determine significance, alpha level was set at $p = .05$.

#### 2.3.1. Biological data

First, as a quality control measure, we compared cortisol at the first saliva sample (wake-up) across all subjects and visits, as a significant difference in cortisol at wake-up could be an indicator for failure to have immediately collected the first sample upon awakening [13], at which no difference indicates proper sampling across all subjects.

We then derived two distinct metrics from the cortisol data: the cortisol awakening response (CAR) and the cortisol amplitude (CAmp). The CAR was analyzed as described previously [3] utilizing a repeated-measures ANOVA on the fitted data of the five morning samples (for *timepoints* wake-up until 60 min post-awakening).

The CAmp was then calculated as the difference between the morning peak and the lowest baseline level in the afternoon. Cortisol secretion follows a circadian cycle, characterized by a wave form with a peak and a trough [28]. When dealing with waves, peak-to-trough amplitude is a straightforward standard measure. The CAmp thus reflects the diurnal amplitude of cortisol with respect to baseline levels [28]. Fig. 1 illustrates this analysis.

For all analyses, covariates included the duration of symptoms (in months), age (in years), sex (dichotomous), smoking (dichotomous), wake-up time, depression (Beck's Depression Inventory [BDI] score), state anxiety (State-Trait-Anxiety Inventory [STAI-S] score), total childhood trauma questionnaire (CTQ) score, and menopause (dichotomous) [29].

#### 2.3.2. Cortisol as a biomarker for FND

First, to evaluate CAR differences across the two *visits* (M0 and M8), a repeated-measures ANOVA was used on the fitted data using a linear mixed model with fixed effects of factor *visit* and *timepoint*, corrected for covariates of no-interest. Post-hoc multiple pairwise comparisons on the adjusted cortisol means between the two *visits* were then performed to identify changes in total cortisol levels per *timepoint*.

Second, for assessing CAmp differences between the two *visits*, we applied an ANOVA on the fitted data of the CAmp using a linear mixed model with a fixed effect of factor *visit* corrected for covariates of no-interest.

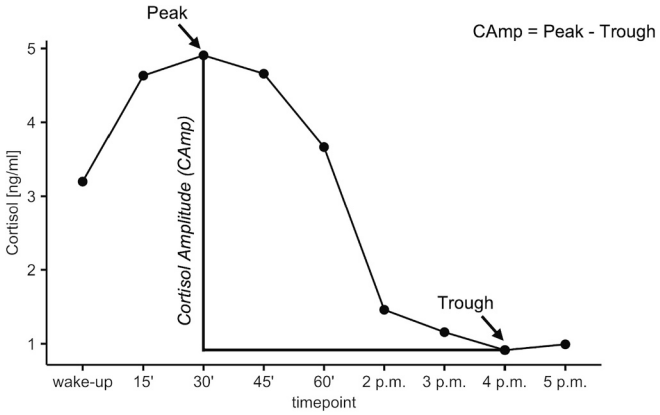


**Fig. 1.** Calculation of the Cortisol Amplitude (CAmp).
Identify the cortisol peak and trough. The CAmp is then further calculated by subtracting the trough from the peak.

Third, we assessed CAR differences between the two *visits* comparing the two clinical outcome *groups* (i.e., objective/subjective improvement versus aggravation/unchanged). This analysis was repeated using fixed effects of factor *group*, *visit* and *timepoint*. We then performed post-hoc multiple pairwise comparisons on the adjusted cortisol means investigating the effect of *clinical outcome group* across the two *visits*, which allows for detecting changes in total cortisol levels per *timepoint* between *groups* and *visits*. Additionally, a post-hoc test compared individual timepoints of CAR peak between patients with clinical improvement and those with aggravation or no change after follow-up (M8). This comparison used a non-parametric Fisher's exact test on the frequencies of CAR peak across the five timepoints, enabling the detection of differences in the temporal dynamics of the CAR peak.

Forth, to evaluate the association between CAmp and the two clinical outcome *groups*, we applied ANOVAs on the fitted data of the CAmp using a linear mixed model with fixed effects of factor *visit* and *group*, respectively.

#### 2.3.3. Cortisol as a prognostic marker

Lastly, to assess whether the CAR might serve as prognostic marker for clinical outcome in FND, we implemented a predictive model using a general linear regression model. As such, we tested whether symptom severity at M8 could be predicted by 1) the CAR or 2) the CAmp at M0. We additionally investigated the prognostic abilities of the following covariates: duration of symptoms, age, sex, BDI, STAI-S, total CTQ score.

## 3. Results

### 3.1. Clinical and demographic characteristics

The most common symptom presentations were sensorimotor deficit (49%), gait disorder (32%), and/or functional seizures (15.1%), Table 1. Our quality control analysis to check for possible bias due to dropout showed that patients who did not terminate the study, had higher state-anxiety scores ($t(66.4) = -2.11$, $p = .03$, Supplementary Table 1).

One patient was excluded for analyses involving the CAR due to significant delays for the morning samples (at a strict sampling accuracy margin of $\Delta t > 5$ min [13]). This patient was not excluded for analyses on CAmp, as the CAmp is not particularly affected by sampling delays. Adherence to the protocol regarding confounding factors (e.g., smoking) was orally confirmed with the patients. All patients adhered properly regarding confounding factors. Our quality control analysis on sampling timepoint showed that no significant differences were detected in the first measurement timepoint, indicating proper sampling.

Patients did not significantly differ in depression, anxiety, and symptom severity (S-FMDRS, CGI-S) between M0 and M8, see Table 2. Upon our stratification based on symptom severity assessed using $\Delta_{S\text{-}FMDRS}$, $\Delta_{CGI\text{-}S}$ and CGI-I, 31 patients were categorized as objectively clinically improved, whereas 22 patients were categorized as worse/unchanged. Upon our stratification based on subjective general health ($\Delta_{SF\text{-}36}$), 15 patients were categorized as subjectively improved and 38 as subjectively aggravated/unchanged, see Supplementary Fig. 1 and Supplementary Tables 2–4.

### 3.2. Cortisol as a biomarker for FND

Diurnal cortisol levels (including CAR) between the two visits are illustrated in Fig. 2. First, when investigating the change in CAR across the two visits, the model demonstrated no significant effect of *visit,* suggesting no significant alterations in secreted cortisol levels. Second, for the CAmp, no significant effect of *visit* was detected, indicating no significant differences in CAmp across the two visits.

Third, we assessed CAR differences between the objective and subjective clinical outcome groups (Table 2). First, no significant effects were found upon stratification into objective clinical outcome. The post-hoc multiple pairwise comparisons revealed no statistically significant differences in adjusted cortisol means across the two groups and visits. Within the framework of an additional post-hoc test, non-parametric Fisher's exact test on the frequencies of CAR peak across the five *timepoints* was calculated to investigate cortisol differences between groups across the five samples. The results of the Fisher's exact test indicated a significant association between objective clinical outcome group ($p = .013$, odds ratio (OR): 1.78 and 95% confidence interval (CI): 0.095–1.13) and *timepoint of peak* denoting that patients with a clinical improvement at M8 peaked significantly earlier than patients who aggravated or remained unchanged, indicating that clinical improvement is associated with an earlier peak in the CAR rather than a change in total secreted cortisol levels.

For the subjective clinical outcome, we identified a significant interaction between *visit* and *group* ($F(1,503) = 12.95$, $p = .0003$), but did not find significant effects of *visit* nor *group*. The post-hoc multiple pairwise comparisons showed no statistically significant differences in adjusted cortisol means across the two groups and visits, indicating that this effect is not explained by changes in total secreted cortisol. The non-parametric Fisher's exact post-hoc test on the frequencies of CAR peak across the five *timepoints* showed a trend for the association between *timepoint of peak* and subjective outcome group potentially explaining

**Table 1**
Demographic and clinical data ($N = 53$).

| Sample characteristics | |
|---|---|
| Age, mean (SD), years, [range] | 38.06 (14.5)<br>[19–77] |
| Sex (females/males), [%] | 38/15<br>[72% females, 28% males] |
| Hormonal contraception (yes/no)[a] | 13/25 |
| Menopause (yes/no)[a] | 6/32 |
| Menstrual cycle[a] | 7 anovulation<br>8 follicular<br>17 luteal<br>2 menstruation<br>4 ovulation |
| Smoker (yes/no) | 21/32 |
| Duration of illness, mean (SD), in months | 56.8 (60.53) |
| Symptom type[b] | 26 sensorimotor<br>17 gait disorder<br>8 seizures<br>7 tremor<br>7 myoclonus<br>6 PPPD<br>5 speech disorder<br>4 dystonia<br>1 functional vision loss |
| ICD-10 classification[b] | 37 F44.4<br>4 F44.5<br>20 F44.6<br>5 F44.7<br>6 PPPD |
| Psychotropic medication | 8 benzodiazepines<br>19 antidepressants<br>4 neuroleptics<br>5 antiepileptics<br>4 opioids |
| Psychotropic medication intake (yes/no)[c] | 33/20<br>[62.3% yes, 37.7% no] |
| Corticosteroids (yes/no)[d] | 3/50 |
| CTQ total score, mean (SD) | 43.5 (17.5) |

Abbreviations: CTQ, Childhood Trauma Questionnaire; PPPD, persistent postural-perceptual dizziness; ICD-10, International classification of diseases version 10.

[a] Details on menstrual cycle were only assessed at M0.

[b] Diagnosis of mixed FND (F44.7) was given when F44.4, F44.5, and F44.6 were present. Patients can present with several symptom types.

[c] Out of the 33 patients currently under psychotropic medication, 22 patients took benzodiazepines ($N = 3$ [1.6%]), anti-depressants ($N = 14$ [7.4%]) or a combination of both ($N = 5$ [2.7%]), which potentially affects the HPA-axis.

[d] Corticosteroid medication was only used in a topical form (nasal spray) applied irregularly on demand (not applied on the day of measurement).

**Table 2**
Clinical course at inclusion and after eight months follow-up ($N = 53$).

| Outcomes on clinical course | M0 | M8 | Statistics |
|---|---|---|---|
| Objective clinical outcome (improved/worse or unchanged) | – | 31/22 (58.5% / 41.5%) | |
| Subjective clinical outcome (improved/worse or unchanged) | – | 15/38 (28.3% / 71.7%) | |
| Disease severity (CGI-S, mean, SD) | 2.62 (1.63) | 2.81 (1.66) | $Z = -0.58$, $p = .56$ |
| Disease improvement (CGI-I, mean, SD) | – | 3.74 (1.13) | |
| Disease severity (S-FMDRS, mean, SD) | 8.02 (9.10) | 8.91 (10.8) | $Z = -0.03$, $p = .97$ |
| BDI score, mean (SD) | 13.1 (9.75) | 12.3 (10.7) | $Z = -0.03$, $p = .28$ |
| STAI-S score, mean (SD) | 35.2 (10.6) | 36.3 (11.0) | $Z = -0.49$, $p = .62$ |
| SF-36 General Health, mean (SD) | 49.2 (20) | 51.9 (23.4) | $Z = -0.69$, $p = .49$ |

Data were tested for normal distribution using Shapiro-Wilk's test, and further analyzed using paired parametric or non-parametric tests, respectively.
Abbreviations: M0, at inclusion; M8, at follow-up; S-FMDRS, functional movement disorder rating scale; CGI-S, clinical global impression score; CGI-I, clinical global improvement score; BDI, Beck's depression inventory; STAI-S, State-trait anxiety inventory for state.

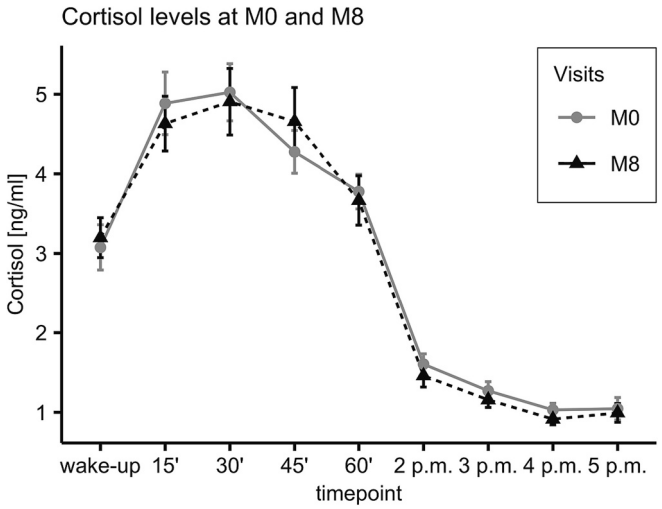


**Fig. 2.** Longitudinal cortisol measures in functional neurological disorders (FND).
Diurnal cortisol levels in patients with FND at the first visit (M0) and at the follow-up examination after eight months (M8). The first five measures (wake-up until 60′) represent the cortisol awakening response (CAR), the last four measures represent diurnal baseline cortisol levels.

the significant interaction term ($p = .08$, OR: 1.34, 95%-CI: −0.20 - 0.84). Individual peak timepoints at M8 are depicted in Table 3. The graphs based on the two different stratifications are shown in Fig. 3.

Forth, no effect of *group* nor *visit* was identified on the CAmp.

### 3.3. Cortisol as a prognostic marker

Objective clinical outcome at M8 was significantly predicted by CAmp at M0 ($\beta = 1.14$, 95%-CI:0.15–2.13, $p = .03$), with a lower CAmp at M0 predicting lower symptom severity (S-FMDRS) at M8. None of the other measures nor any of the covariates individually at M0 significantly predicted clinical outcome at M8.

**Table 3**
Peak in Cortisol Awakening Response (CAR) at M8, showing the number of subjects who peaked at the respective timepoints for the objective and subjective outcome stratification.

| | Objective stratification | Subjective stratification |
|---|---|---|
| Total number of patients per group [improved/aggravated] | 31 / 22 | 15 / 38 |
| Peak timepoint [improved/aggravated] | | |
| Wake-up | 6 / 4 | 2 / 8 |
| 15′ | 8 / 3 | 6 / 5 |
| 30′ | 13 / 4 | 6 / 11 |
| 45′ | 3 / 6 | 0 / 9 |
| 60′ | 0 / 5 | 1 / 4 |
| Statistical difference | Two-sided, $p = .01$ * | Two-sided, $p = .08$ • |
| Fisher's exact test | OR: 1.78 95% CI: 0.095–1.13 | OR: 1.34 95% CI: −0.20 - 0.84 |

Abbreviation: OR = Odds ration; CI = confidence interval.
Statistical significance: $p \bullet < 0.1$, $p^* < 0.05$.
Results of Fisher's exact test result from a 5 × 2 contingency table with number of peaks at the 5 different timepoints for the 2 outcome groups.

## 4. Discussion

Our study firstly examined the potential of cortisol to serve as a clinical marker for FND in a longitudinal design. Using two cortisol measures (CAR and CAmp), we investigated potential changes in cortisol longitudinally and assessed the association between cortisol and clinical outcome. Additionally, we explored the prognostic abilities of cortisol by stratifying patients based on their clinical outcome after eight months.

### 4.1. A flattened CAR represents a trait marker for FND

First, no changes in the CAR nor the CAmp were identified between the two visits at M0 and M8. Thus, even after eight months, patients maintained a flattened CAR, supporting the previous notion that a flattened CAR might represent a *trait* marker for FND [3], potentially associated with a preceding maladaptive downregulation of the HPA-axis in response to prolonged stress [16]. This is the first study investigating cortisol in a longitudinal design in patients with FND. Similar patterns have been observed in trauma survivors who developed PTSD, showing an initial cortisol hyperreactivity to stress upon trauma exposure, followed by a flattened cortisol response at a three- and six-months follow-up regardless of their PTSD status [30], indicating a long-term downregulation of the HPA-axis [31], which has also been suggested in our previous work [3]. However, further longitudinal studies on FND patients, their clinical outcome of patients and associated biomarkers must be conducted to confirm the herein presented results.

### 4.2. An earlier peak in CAR represents a state marker for FND

We showed that when patients improved in their symptom severity after eight months, they appeared to peak earlier (but not higher) in their CAR compared to patients with a worse or unchanged outcome. Thus, a clinical improvement is reflected by means of an earlier peak in the CAR, and not by a change in total secreted cortisol. This state-dependent change in the CAR may indicate that timepoint of peak potentially represents a *state* marker for FND. However, previous studies investigating on the effect of symptom severity on the CAR in FND did not detect any correlations between symptom severity and the CAR (collected at wake-up and 30 min post, domestic setting [32]; measured at wake-up and 30 min post, hospitalized overnight [17], assessed using e.g., AUC [33] as an approximation to the CAR). However, as the effect of clinical outcome seems to be associated with the timepoint of peak

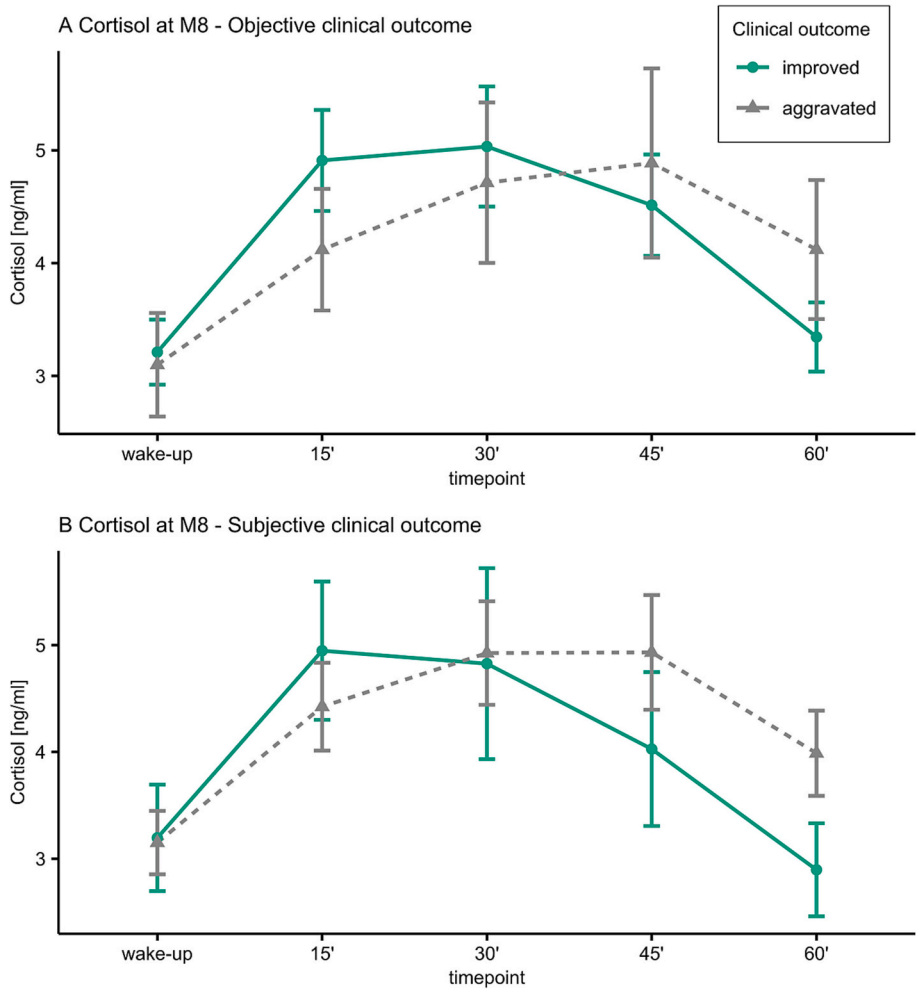


**Fig. 3.** Cortisol Awakening Response (CAR) and clinical outcome in functional neurological disorders (FND).
(A) CAR at M8 in patients stratified into objective clinical improvement ($N = 31$) and aggravation/ no change ($N = 22$) based on changes in the functional movement disorders rating scale (S-FMDRS), changes in the clinical global impression (CGI-S) scale and the clinical global improvement scale (CGI-I) at M8. Patients with an improved clinical outcome at M8 peaked earlier ($p = .013$) in comparison to those who aggravated or remained unchanged. (B) CAR at M8 in patients stratified into subjective clinical improvement ($N = 15$) and aggravation/ no change ($N = 38$) based on the minimal clinical important difference (MCID) in the SF-36 subscale of general health. A significant interaction between visit and group was identified ($F(1,503) = 12.95$, $p = .0003$), for which a trend was found in post-hoc tests on peak timepoint with improved subjects tending towards an earlier peak ($p = .08$, OR: 1.34, 95% CI: −0.20 - 0.84).

rather than a change of total secreted cortisol (as measured using AUC), previous analyses with two samples might not have been sensitive enough to capture a difference in peak timepoint associated with symptom severity. Accordingly, [14] previously determined in healthy controls that the peak of CAR occurs between 15 and 30 min after awakening, rather than a discrete CAR peak at one-time point without making further interpretation of its biological importance. Similarly, it could be shown that a three versus five timepoint sampling protocol resulted in a slightly lower accuracy and precision of the analyses [34]. Future studies investigating on dynamic changes in the CAR would benefit from sufficient sampling timepoints to capture not only changes in cortisol levels but also in timepoint of peak as a potential state marker for FND [13,33].

### 4.3. Cortisol amplitude reflects a prognostic marker for clinical outcome

We demonstrated that the CAmp at M0 predicts objective clinical outcome at M8, offering a potential *prognostic* biomarker for FND. This application of the peak-to-trough wave amplitude on the CAR, as introduced by Evans [28], was suggested to reveal trait-like associations such as an underlying vulnerability. In contrast, AUC-based measures capture state-like influences on the CAR. Evans [28] further invited to reevaluate the over-simplified dichotomic concept of viewing a clinical disorder as being either hypo- or hyper-expressing by returning to understand the CAR as a complex and dynamic biological phenomenon rather than a simple response to "waking-up". Future studies in FND should longitudinally observe the CAR, considering its amplitude and flexibility concerning clinical outcomes when analyzing cortisol data [28,35]. These approaches may contribute to a better understanding of the incongruity among previous results [3,17,32] and advocate to examine diverse composites of the CAR to untangle its biological significance in FND. Properly applied and validated, the CAR – and its derivative, the CAmp – could serve as a prognostic biomarker, identifying patients with a poor outcome who would benefit from more frequent clinical follow-ups, or helping monitor disease progression and treatment outcomes.

### 4.4. Potential biological implications of the CAR

Understanding the biological implications and regulations of the cortisol awakening response (CAR) is crucial, as its exact role in neurobiological processes, whether in health or disease, remains incompletely explained. The prevailing theory suggests that the CAR activates prospective memories in the hippocampus, aiding in

orientation in time and space, and anticipates imminent systemic demands for the forthcoming day [36,37]. A higher CAR has been associated with increased needs of energy [38], i.e., when a stressful day is ahead, which requires more resources. Hence, the hippocampus (along with the prefrontal cortex (PFC) and the amygdala) is hypothesized to be the main regulatory region of HPA-axis activity [39], including the CAR [40].

As example, a higher CAR was associated with a reduced sustained attention towards aversive stimuli [41]. Recent work investigated the role of the CAR on functional brain networks suggesting that the CAR indeed plays a causal role in functional coordination of top-down modulation of the dorsolateral PFC to the hippocampus supporting the theory of the brain's preparedness in anticipation of upcoming demands [42]. In the context of FND, patients are proposed to be hypervigilant towards threat [43], leading to constant anticipation of increased levels of stress based on previous aversive experiences [44]. Consequently, patients might constantly anticipate higher metabolic needs, eventually resulting in HPA-axis downregulation [3] and the body redirecting towards a sickness behavior (e.g., fatigue) with the aim to reduce predicted energy expenditures [45]. In line with this, chronic stress and an HPA-axis downregulation may be directly related to a reduced adrenocorticotropic-hormone (ACTH) sensitivity [46], possibly explaining the delayed peak in the CAR in patients with more severe symptoms. Likewise, a slower cortisol response upon ACTH stimulation has been linked to higher susceptibility to stress in [47].

In summary, the here found results might 1) provide evidence to the maladaptive downregulation of the HPA-axis in FND patients (possible *trait* biomarker as irrespective of clinical improvement at M8 the CAR is flattened), and 2) provide utility regarding its association with clinical improvement (*state* biomarkers as timepoint of earlier peak was associated with better clinical outcome) and 3) serve as indicator to the prognosis of clinical outcome (*prognostic* biomarker as the CAmp at M0 could predict outcome at M8).

### 4.5. Limitations

Several limitations should be considered. Firstly, salivary cortisol collection within a domestic environment relies on self-reported diaries and on patients reporting deviations (e.g., smoking during sampling periods) accordingly. These reports were also double-checked orally with the investigator and patients appeared to adhere well to the protocol. However, as this is based on self-reported data, we cannot fully exclude non-adherence. Objective verification of awakening time could further help identifying inaccurate sampling [48]. Moreover, we did not collect data on menstrual cycle, contraception and medication intake at follow-up which did not allow us to correct for this in our results. This is of great interest for future studies, as especially benzodiazepines and certain anti-depressants might interact with the HPA-axis. Secondly, the stratification into clinical outcome in FND patients can be challenging as no clear consensus guidelines exist [49]. To consider not only objective physical symptoms, but also subjective perception of well-being, we applied two different stratifications, which lead to different numbers of patients assigned to the respective groups highlighting the importance of objective but also subjective impression of clinical outcome. However, our stratification is in line with previous reports [49] on that only around 20% of patients show a clinical improvement at a follow-up. Likewise, we must acknowledge that our two stratification approaches are clinician/examiner-rated versus patient-rated, which does not necessarily overlap with subjective/objective as patient-rated measures are normally subjective while clinician/examiner-rated measures can be either. Nevertheless, a complementary set of personalized self-reported (subjective) and objective (examiner-rated) outcome measures are recommended for FND [50]. Another limitation of our study lied in the fact that the scores we used to assess improvement are scales mainly used in research which may not fully reflect a meaningful clinical improvement. There is currently no consensus on what the best outcome measure for FND is [50] but international initiatives are underway to propose such outcome measures for future work. Thirdly, functional symptoms can be very fluctuating and therefore, a clinical improvement might only be temporary and might not represent disease progression. Also, we chose a follow-up window at eight months, but results may be different in a more prolonged time, as HPA-axis modulation may take longer to adapt than clinical recovery. In addition, we included mostly patients with chronic symptoms (4.7 years). Only further long-term longitudinal studies may provide a definite answer regarding the question of state/trait values of cortisol findings in FND. Fourthly, a transdiagnostic approach was applied with patients presenting with various symptoms and potential psychiatric comorbidities. A change of the CAR with respect to clinical improvement has also been identified in other psychiatric disorders [19,20] [21,22], and thus, might not be specific to FND. The herein reported results could represent various degrees of underlying (untreated) mental health disorders which represents a poor prognostic factor for outcome [51]. Thus, as a major limitation, we lack a systematic psychiatric evaluation and thus, other psychiatric comorbidities, which are common in FND [52], might have remained undetected. Even though we excluded patients with other major psychiatric disorder, we must note that our patients had relatively high total CTQ scores, thus, we might have missed an underlying diagnosis of PTSD (subclinical or partially recovered PTSD). This might be of importance, as PTSD patients also showed a flattened cortisol response at follow-up regardless of their clinical status [30]. Similarly, the 33 patients who did not complete M8 were found to be more anxious at M0 compared to those patients who finished the study. As most of the reasons were not related to FND, we cannot exclude that patients who withdrew consent or were lost for follow-up would have affected the results differently. In line with this, the study had a very high dropout rate, which represents one of the major limitations. These dropouts could have recovered and left services or, conversely, not improved, possibly deteriorating and missing the follow-up. Apart from reducing the statistical power, such a selection bias might affect the generalizability of the results, or an overestimation of the true effect. While this limitation not only highlights the need for the replication of our previous [3] and current work, but it also emphasizes the necessity of studies including psychiatric and neurological control patients.

## 5. Conclusion

In summary, we confirmed previous results [3] showing that FND patients display a flattened CAR that stays low at eight-month follow-up, suggesting the flattened CAR to possibly represent a *trait* marker for FND. Furthermore, we showed that an improvement in symptom severity at M8 was associated with an earlier peak in the CAR at M8, which might serve as a potential *state* marker of FND. Finally, the CAmp at M0 successfully predicted clinical outcome after eight months and therefore might serve as a *prognostic* marker for FND. To conclude, cortisol – and from it derived the CAR and CAmp – might be considered a biomarker for neuropsychiatric patients such as FND, but further research is required to disentangle the underlying biological significance as well as the contribution of underlying psychiatric comorbidities.

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## Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT 3.5 in order to spell check and proof-reading assistance. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

## CRediT authorship contribution statement

**Samantha Weber:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Janine Bühler:** Writing – review & editing, Project administration, Investigation, Data curation. **Fabian Messmer:** Writing – review & editing, Methodology, Conceptualization. **Rupert Bruckmaier:** Writing – review & editing, Resources, Funding acquisition. **Selma Aybek:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors have no competing interests to report.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychores.2024.111615.

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