# Emotional conflict processing in adolescent Chronic Fatigue Syndrome: a pilot study using *f*MRI

**Laura Anne Wortinger[1, 2*], Tor Endestad[2], Annika Melinder[3], Merete Glenne Øie[2, 4], Dag Sulheim[5, 6], Even Fagermoen[7], and Vegard Bruun Wyller[1]**

[1]*Department of Pediatrics, Akershus University Hospital, Nordbyhagen, Norway*
[2]*Department of Psychology, University of Oslo, Norway*
[3]*Cognitive Developmental Research Unit, Department of Psychology, University of Oslo, Norway*
[4]*Research Department, Innlandet Hospital Trust, Lillehammer, Norway*
[5]*Department of Pediatrics, Oslo University Hospital, Norway*
[6]*Department of Pediatrics, Innlandet Hospital Trust, Lillehammer, Norway*
[7]*Department of Anesthesiology and Critical Care, Oslo University Hospital, Norway*

[*] Address for correspondence:
Laura Anne Wortinger, Department of Psychology, University of Oslo, PO Box 1094 Blindern, 0317 Oslo, Norway.
Email: l.a.w.bakke@medisin.uio.no

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*Introduction*: Studies of neurocognition suggest abnormalities in cognitive control contribute to the pathophysiology of chronic fatigue syndrome (CFS) in adolescents, yet these abnormalities remain poorly understood at the neurobiological level. Reports indicate that adolescents with CFS are significantly impaired in conflict processing, a primary element of cognitive control. *Method*: In this study, we examine whether emotional conflict processing is altered on behavioral and neural levels in adolescents with CFS and a healthy comparison group. Fifteen adolescent patients with CFS and 24 healthy adolescent participants underwent functional magnetic resonance imaging (*f*MRI) while performing an emotional conflict task that involved categorizing facial affect while ignoring overlaid affect labeled words. *Results*: Adolescent CFS patients were less able to engage the left amygdala and left mid-posterior insula (mpINS) in response to conflict compared to the healthy comparison group. An association between accuracy interference and conflict-related reactivity in the amygdala was observed in CFS patients. A relationship between response time interference and conflict-related reactivity in the mpINS was also reported. Neural responses in the amygdala and mpINS were specific to fatigue severity. *Conclusions*: These data demonstrate that adolescent CFS patients displayed deficits in emotional conflict processing. Our results suggest abnormalities in affective and cognitive functioning of the salience network, which might underlie the pathophysiology of adolescent CFS.

**Key words:** chronic fatigue syndrome; adolescents; functional MRI; cognitive control; emotion; conflict

## Introduction

Disabling physical and mental fatigue, which worsens from physical and mental exertion, characterize chronic fatigue syndrome (CFS) (IOM, 2015). CFS constitutes one of the major threats towards adolescent health (Royal College of Paediatrics and Child Health, 2004). Neuropsychological studies have documented cognitive impairments in adolescent patients, suggesting that a sustained stress response might be an important part of the pathophysiology (Wyller, Eriksen, & Malterud, 2009). Recently, a deficit in cognitive inhibition was reported in adolescents with CFS (Sulheim et al., 2015). In another study, the Eriksen Flanker test revealed conflict processing impairment in adolescents with CFS (van de Putte et al., 2008). Haig-Ferguson, Tucker, Eaton, Hunt, and Crawley (2009) and Kawatani et al. (2011) reported attention impairments in adolescent CFS patients. An event-related potential (ERP) study found working memory impairment was associated with frontal lobe alterations in adolescents with CFS using KANA-Pick-out test (Tomoda et al., 2007). These studies point to a specific impairment in cognitive control and warrant further investigation.

The human cognitive system has the remarkable ability to adapt efficiently and effectively to a changing environment. This would entail an efficient salience network (SN) in the brain that identifies and integrates salient events, both endogenously and externally cued, to help guide behavior. The main regions of the brain that comprise the SN are the insula, dorsal anterior cingulate cortex (dACC), and amygdala. The dynamic hub of the SN is the fronto-insular cortex (FIC) that integrates other SN regions in the processing of sensory, emotional and cognitive information (Menon, 2015). Importantly, the right FIC has been implicated as the region of the SN, which mediates autonomic signaling with conscious awareness (Craig, 2002; Critchley, 2004; Critchley, Melmed, Featherstone, Mathias, & Dolan, 2002). The worsening of symptoms after physical and mental exertion (post-exertional malaise), as seen in CFS,

could suggest a malfunction of the SN, because inefficiencies might demand greater mental effort and disrupt the interpretation of salient biological and cognitively important information.

SN theory suggests that deficiencies in the in filtering and registering of salient stimulus cues into the SN influence the engagement of other brain networks, such as the lateral frontoparietal network, which leads to impoverished cognition (Menon, 2015). Previous neuroimaging studies on adults with CFS have shown functional and anatomical alterations in brain areas with reduced activity in the dorsolateral and medial prefrontal cortex, anterior cingulate gyrus, insula, and parietal cortices (Caseras et al., 2006; Caseras et al., 2008; de Lange et al., 2004) during cognitive tasks, and decreased grey-matter volume in bilateral prefrontal cortices (de Lange et al., 2008; Okada, Tanaka, Kuratsune, Watanabe, & Sadato, 2004). A recent *f*MRI study on childhood CFS found that patients exhibited less efficient frontal activity during a dual verbal task, where increased mental effort afforded costly energy requirements (Mizuno et al., 2015). Fatigue influences neural function and studies relating these changes to the specificity of fatigue and not to confounding factors like anxiety and depressive symptoms are currently missing in the literature.

In a recent review on predisposing, precipitating, and perpetuating factors in adolescent CFS, an eminent finding was the higher rate of comorbidity with anxiety and depression disorders compared to healthy controls or illness control groups (Lievesley, Rimes, & Chalder, 2014). Anxiety and depression are indicators of altered emotional processing and also a negative affect bias, in terms of impaired performance to faces expressing negative emotion (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van, 2007; Gotlib, Krasnoperova, Yue, & Joormann, 2004). However, studies focusing on how emotional processing interact with underlying brain regions and their associated cognitive functions have not been undertaken previously in adolescents with CFS.

The cognitive ability to detect a conflict and configure a response for successful conflict resolution has been operationalized in the context of the Stroop task (Botvinick, Braver, Barch, Carter, & Cohen, 2001; Kerns et al., 2004; MacDonald, Cohen, Stenger, & Carter, 2000). Conflicting stimuli in a Stroop task produce an interference in cognitive processing that can be measured behaviorally by increases in response time and decreases in accuracy. An *emotional* Stroop task was designed to explore how emotion-laden stimuli interact with cognitive control and might therefore provide important knowledge on adolescent CFS pathophysiology. When this task was performed on healthy adults, the medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC), and amygdalae have been implicated in conflict detection, stimuli appraisal, and regulation (Egner, Etkin, Gale, & Hirsch, 2008; Etkin, Egner, Peraza, Kandel, & Hirsch, 2006). However, the detrimental effects of interpersonal stress appeared to alter activity in the right FIC and left mid-posterior insula (mpINS) during an emotional conflict task (Bruce et al., 2012; Marusak, Etkin, & Thomason, 2015). CFS appears to be strongly associated with childhood adversities (Afari et al., 2014), and the underlying pathophysiology is indicative of a sustained stress response (Wyller et al., 2009), adding to the possible usefulness of applying the emotional Stroop task in an experimental design.

The aim of this study was to explore and link emotional conflict processing to underlying neural mechanisms in adolescent CFS. To gauge emotional conflict processing, we measured the amount of interference, response time slowing and decrease in accuracy, observed on behavioral measures. Firstly, we hypothesized adolescent CFS patients would show reduced behavioral conflict interferences. Secondly, we hypothesized that adolescent CFS patients would exhibit decreased responses in conflict detection regions of the SN: ACC, amygdalae, and insula, and that these alterations would be related to behavioral interferences. Finally, we explored associations between depressive and anxiety symptoms and neural function and

tested whether adolescent CFS patients would show a negative affect bias, a common marker in mood and anxiety disorders.

## Material and Methods

This study is part of the NorCAPITAL-project (The Norwegian Study of Chronic Fatigue Syndrome in Adolescents: Pathophysiology and Intervention Trial) (Clinical Trials ID: NCT01040429). It was conducted at the Department of Pediatrics, Oslo University Hospital, Norway, which is a national referral center for young CFS patients. The current study is based on cross-sectional data collected during the first clinical in-hospital day of NorCAPITAL, from March 2010 to May 2012. All participants received a gift-card worth NOK 200. Informed, written consent was obtained from all participants and from parents/next-of-kin if required. The study was conducted in accordance with the Helsinki Declaration of the World Medical Assembly and approved by the Norwegian National Committee for Ethics in Medical Research.

### *Participants*

All hospital pediatric departments in Norway (n=20), as well as primary care pediatricians and general practitioners, were invited to refer CFS patients aged 12-18 years consecutively to our department.

The referring units were equipped with written information for distribution to potential study participants and their parents/next-of-kin. If consent was given, a standard form required the referral unit to confirm the result of clinical investigations considered compulsory to diagnose pediatric CFS (pediatric specialist assessment, comprehensive hematology and biochemistry analyses, chest x-ray, abdominal ultrasound, and brain magnetic resonance imaging)(Royal College of Paediatrics and Child Health, 2004). Also, the referring units were required to confirm that the patient a) was unable to follow normal school routines due to fatigue; b) was

not permanently bedridden; c) did not have any concurrent medical or psychiatric disorder that might explain the fatigue; d) did not experience any concurrent demanding life event (such as parents' divorce) that might explain the fatigue; e) did not use pharmaceuticals (including hormone contraceptives) regularly. If medical history or current health status indicated a psychiatric condition, physicians were required to refer patients to a psychiatrist for evaluation. If a comorbid psychiatric disorder was found, those patients were removed from the study (Sulheim et al., 2014). No patients received graded exercise therapy (GET) and two patients (out of the 15 viable *f*MRI datasets) received cognitive behavioral therapy (CBT) at baseline. Completed forms were consecutively conveyed to the study center and carefully evaluated by either of two authors (DS or EF). Patients, considered eligible for this study, were summoned to a clinical meeting at our study center, and after which, a final inclusion decision was made.

In agreement with NICE clinical guidelines (Royal College of Paediatrics and Child Health, 2004; National Institute for Health and Clinical Excellence, 2007), we applied a 'broad' case definition of CFS, requiring three months of unexplained, disabling chronic/relapsing fatigue of new onset. We did not require that patients meet any other accompanying symptom criteria, in contrast to the case definition from the International Chronic Fatigue Syndrome Study Group at the Centers for Disease Control and Prevention (commonly referred to as the Fukuda-definition), which appears to be most frequently used in the scientific community (Fukuda et al., 1994). The Fukuda-definition requires at least six months of unexplained chronic or relapsing fatigue of new onset, severely affecting daily activities, as well as four or more of eight specific accompanying symptoms (headache, muscle pain, joint pain, sore throat, tender lymph nodes, impaired memory or concentration, unrefreshing sleep, and malaise after exertion). However, the validity of this definition has not been established (Brurberg, Fonhus, Larun, Flottorp, & Malterud, 2014). In fact, several empirical findings

raise concerns about the validity, in particular among adolescents: A formal factor analysis of symptoms in a broadly defined group of chronic fatigued patients did not show a strong correspondence with the Fukuda accompanying symptoms (Nisenbaum, Reyes, Unger, & Reeves, 2004). A study based upon the Swedish twin registry concluded that there was no empirical support for the requirement of four out of eight Fukuda accompanying symptoms (Sullivan, Pedersen, Jacks, & Evengard, 2005). A report on a broadly defined population of adolescent CFS patients concluded that the subgroup adhering to the Fukuda criteria was not characterized by a certain level of disability, nor was this subgroup specifically related to characteristics of underlying pathophysiology (alteration of cardiovascular autonomic control) (Wyller & Helland, 2013). Accordingly, subgrouping based upon the Fukuda criteria did not influence the cross-sectional comparisons or the intervention effects in previously reported results from the NorCAPITAL project (Sulheim et al., 2014). Thus, the inclusion criteria in this study are wider than the Fukuda criteria. The main reason for not adhering to the Fukuda case definition was too few accompanying symptoms. Disease duration and percentage of patients fulfilling Fakuda and NICE criteria were reported.

In NorCAPITAL, a total of 120 CFS patients were included. This study was based upon a subset of patients generated from a computer-based randomization procedure, where one fourth of the patients were randomized to be included in the present study (Sulheim et al., 2014). Disease mechanism in CFS might change over time; in addition, disease duration might be a marker of prognosis. Thus, 18 months disease duration (median disease duration in the NorCAPITAL cohort) served as stratification criterion for the randomization procedure (Sulheim et al., 2014). The randomization procedure allocated 30 patients to fMRI assessment: of these, five patients did not want to participate in the study, four patients were excluded due to orthodontic treatment, three patients were excluded due to excessive movement parameters > 3 mm in either of the three translation parameters or three rotation parameters, one was

209 excluded due to poor performance (<50% accuracy), and two were excluded due to scanning
210 error, resulting in a total fMRI dataset of n = 15 adolescent CFS patients for the final analyses.
211 A group of 24 healthy controls having a comparable distribution of gender and age were
212 recruited from local schools. No chronic disease and no regular use of pharmaceuticals were
213 allowed. All participants completed the Spielberger State-Trait Anxiety Inventory
214 (Spielberger, Gorsuch, & Lushene, 1973), Mood and Feelings Questionnaire for Depression
215 (Sund, Larsson, & Wichstrom, 2001),Wechsler Abbreviated Scale of Intelligence (WASI)
216 (Wechsler, 2007), and Chalder Fatigue Questionnaire (Chalder et al., 1993). Symptom data
217 were missing at random for two of the patients, and the group mean was used for their lost
218 data.

219 ***Clinical Measures***

220 The Chalder Fatigue Questionnaire is a valid outcome measure in adult (Chalder et al., 1993)
221 and adolescent CFS (Tanaka et al., 2008) based on symptoms during the preceding month.
222 The sum across 11 items is scored on a 0-3 Likert scale, thus ranging from 0 (less severe
223 fatigue) to 33 (more severe fatigue).

224 The Mood and Feelings Questionnaire (MFQ) has been validated in children and adolescents
225 (Sund et al., 2001). MFQ consists of 34 items to be rated based on symptoms during the
226 preceding month. Each item is scored on a 0-2 Likert scale, and the total sum score is from 0
227 to 68. Higher scores imply more depressive symptoms.

228 The state anxiety measure from the Spielberger State-Trait Anxiety Inventory (Spielberger et
229 al., 1973) is a valid measure of 12 items that asks participants to indicate how they feel right
230 now on 4-point forced-choice Likert-type response scales. Scores range from 12 to 48, with
231 higher scores suggesting greater levels of anxiety.

232 ***Experimental Paradigm***

233 The emotional conflict task was modified from the previously described paradigm (Egner et
234 al., 2008; Etkin et al., 2006). It consisted of 168 presentations of photographs of happy or
235 fearful facial expressions drawn from the Karolinska database (Lundqvist, 1998). Faces were
236 cropped and overlaid with the words "FRYKT" or "GLEDE," (English: fear or happy,
237 respectively) and written in prominent red letters across the face, such that word and facial
238 expression were either congruent or incongruent trial types (Figure 1). Stimuli were presented
239 using E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA) and MR-compatible
240 goggles with two LCD-displays (VisualSystems®; NordicNeuroLab, Bergen, Norway), while
241 responses were collected using an MR-compatible response grip with two response buttons
242 (ResponseGrip®; NordicNeuroLab, Bergen, Norway). Stimuli were presented for 1000 msec,
243 interstimulus interval with fixation + of 3000 msec, and jitter of 1250-2000 msec (mean ITI =
244 4000) in a pseudorandom order, and counterbalanced across trial types for expression, word,
245 gender and response button. Participants indicated facial affect with a button press response.

246 Participants were instructed to indicate the emotional expression in the face (target), where a
247 word was written across that was either semantically congruent or incongruent with the facial
248 affect. Congruent conditions have semantically compatible faces and words and usually result
249 in better performance, as there is no conflict. The congruent word thus represents a distractor
250 that facilitates cognition. However, an incongruent word stimulus would elicit incompatible
251 response tendencies, one of which is the overlearned prepotent response to read the word. The
252 incongruent word represents a distractor that interferes with cognition and initiates cognitive
253 conflict processing.

254 Behavioral data, accuracy and response times (excluding error trials), were analyzed in SPSS,
255 version 20, (SPSS, Inc., Chicago, IL). Interference in the response time (RT) data was

calculated by subtracting the mean response times of the congruent (C) trials from the mean response times of the incongruent (I) trials. Interference in accuracy (AC) data was also calculated by subtracting mean accuracy scores on congruent trials from mean accuracy scores on incongruent trials. Emotional conflict (I-C) would be indicated on behavioral measures by producing a slowdown in IC-RT and a reduction in IC-AC. Behavioral effects were considered significant at a $p \leq 0.05$ (two-tailed) threshold.

To test for a negative affect bias in both the RT and AC data, a 2 x 2 x 2 facial affect (fear vs. happy) x congruency (congruent vs. incongruent) x group (CFS patients vs. comparison group) repeated measures ANOVAs were used.

### *fMRI Data Acquisition*

Scanning was conducted on a 3 T, Phillips Achieva whole-body scanner, with an 8 channel Philips SENSE head coil (Philips Medical Systems). Functional images were obtained with a single-shot $T_2$* - weighted echo planar imaging sequence (repetition time (TR): 2000 msec; slice echo time (TE): 30 msec; field of view (FOV): 240 x 240; imaging matrix: 80 x 80; flip angle 80° 35 axial slices, interleaved at 3mm thickness, no gap, voxel size 3 x 3 x 3 mm. The scanning session consisted of 510 volumes, synchronized to the onset of the experiment. A $T_1$ - weighted anatomical image with a voxel size of 2 x 2 x 2 mm was recorded for registration of the functional images (60 transverse slices; TR: 10462 msec; TE: 54 msec; FOV: 224 x 224; flip angle: 90°).

### *fMRI Data Analysis*

Functional images were converted to 4D NIfTI files (http://lcni.uoregon.edu/jolinda/MRIConvert/) and analyzed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8). Images were preprocessed using the standard SPM pipeline (Ashburner et al., 2012)- corrected for slice timing, realigned to

correct for residual head movement, coregistered to the segmented anatomical image, and spatially transformed to the Montreal Neurologic Institute coordinate system (K. J. Friston et al., 1995). Images were resampled every 3 mm, and smoothed with an 8 mm full-width - half-maximum (FWHM) Gaussian kernel. A 128-second temporal high-pass filter was applied to the data. Separate regressors for the stimulus events (congruent and incongruent) were created and convolved with a canonical hemodynamic response function. Error trials were modeled separately. Additional regressors of no interest corresponding to the six motion parameters were also included. This model was applied to normalized data in the context of a generalized linear model (Karl J Friston et al., 1994) and submitted to a group level random-effects analyses using two-sample t tests.

We report group differences from separate *a priori* region of interest analyses using both a voxel family-wise error-correction (FWE) and a Bonferroni correction of $p<0.01$ for multiple tests. Regions online during conflict detection, within the SN and commonly recruited in conflict tasks: bilateral amygdala (left, $x = -30$, $y = -6$, $z = -14$; right, $x = 32$, $y = 0$, $z = -12$), dACC ($x = 2$, $y = 32$, $z = 31$), right FIC ($x = 40$, $y = 30$, $z = -7$) were derived from prior work (Egner et al., 2008; Etkin et al., 2006; Marusak, Etkin, et al., 2015). The left mid-posterior insula region ($x = -34$, $y = -10$, $z = 10$) was included because of its association with stress in previous studies (Bruce et al., 2012; Marusak, Etkin, et al., 2015). Regional masks (8 mm radii spheres) were created around each coordinate using the WFU PickAtlas (Maldjian, Laurienti, Kraft, & Burdette, 2003). The reported voxels correspond to standardized Montreal Neurological Institute (MNI) coordinate space. For regions showing group differences, signal was extracted using MarsBar (Brett, Anton, Valabregue, & Poline, 2002) and analyzed in IBM SPSS v.22 to evaluate the relationship of brain activation, task performance, and symptom severity. Effects were considered significant at a threshold of $p \leq 0.05$ (two-tailed).

Two-way ANOVAs were conducted to examine group variance and activity in brain regions of interest on interference variables.

Next, we explored associations between neural function and fatigue severity and tested for influences of anxiety and depressive symptoms on that relationship in linear regression analyses.

Exploratory whole-brain results for the between-group effects of I-C contrasts are also reported at a threshold of $p < 0.005$, cluster minimum = 10 voxels. This threshold was derived from suggested standards for whole-brain comparisons (Lieberman & Cunningham, 2009) and presented for visualization.

## Results

Adolescent CFS patient and comparison groups were well matched for age, gender, body mass index (BMI) and IQ; however, patients scored higher on clinical symptom scales (Table 1).

### *Behavioral*

In the analysis to determine a negative affect bias in the RT data, there was a significant main effect of congruency ($F$[1, 37]=20.79, $p$<0.001). There were no effects for group, either interactions or between-subjects. Since there was no evidence of groups responding differently with the two emotions, further analyses combined emotions across congruency conditions. There was very strong evidence of response time slowing for incongruent trials, compared to congruent trials, in the comparison group (incongruent response time=785 msec [SD=89], congruent response time=748 msec [SD=87]; $t$=5.63, df=23, $p$<0.001). This effect was not observed in the patient group (incongruent response time=749 msec [SD=75], congruent response time=730 msec [SD=77]; $t$=1.66, df=14, $p$=0.120; see Figure 2A).

In the analysis to determine a negative affect bias in the AC data, there was a significant main effect of congruency ($F$[1, 37]=19.04, $p$<0.001). There was no between-subjects effect for group, but there was evidence of an interaction for emotion and group ($F$ [1, 37]=8.62, $p$<0.01). Independent t-tests revealed a significant difference between patients and controls on happy trials only (patients: happy congruent accuracy =90.0% [SD=8.6]; comparison: happy congruent accuracy=95.8% [SD=4.5]; $t$=2.73, df=37, $p$=0.01; patients: happy incongruent accuracy=83.5% [SD=11.8]; comparison: happy incongruent accuracy=90.9% [SD=8.9]; $t$=2.22, df=37, $p$=0.03). There was no 3-way interaction effect for emotion, congruency and group. A negative affect bias was not observed in the accuracy data, and further analyses combined emotions across congruency conditions. There was good evidence of a decrease in accuracy for incongruent trials, compared to congruent trials, in both the CFS group (incongruent accuracy=86.8% [SD=10.0], congruent accuracy=91.1% [SD=7.2]; $t$=-3.10, df=14, $p$=0.008), and comparison group (incongruent accuracy=91.6% [SD=8.7], congruent accuracy=95.2% [SD=5.2]; $t$=-3.17, df=23, $p$=0.004; see Figure 2B).

***Functional MRI***

We examined the neural correlates of group differences on the emotional conflict contrast (I-C) in *a priori* region of interest analyses and found the left amygdala revealed a significant decrease in conflict-related activity in CFS patients compared to the comparison group, $x$ = -27, $y$ = -4, $z$ = -11, $Z$ = 3.45, $p$FWE = 0.009 (Figure 3A). The right amygdala did not show group differences in responses to conflict. CFS patients showed a significant decrease in reactivity of the left mid-posterior insula (mpINS), $x$ = -39, $y$ = -7, $z$ = 7, $Z$ = 3.80, $p$FWE = 0.003 (Figure 3C), in response to conflict compared to the comparison group. Conflict-related activity in the right FIC and dACC did not show group differences.

Left amygdala activity was significantly related to accuracy interference, $F(1, 35) = 4.25$, $p < 0.05$, $\eta_p^2 = .11$ There was also good evidence of an interaction between the effects of group and left amygdala reactivity on accuracy interference, $F(1, 35) = 8.58$, $p < 0.01$, $\eta_p^2 = .20$; Figure 3B). The relationship between conflict-related activity in the left amygdala and IC-AC decreased in the CFS group, but increased in the comparison group.

Group variance was significantly related to response time interference, $F(1, 35) = 4.28$, $p < 0.05$, $\eta_p^2 = .11$. There was also evidence of an interaction between the effects of group and mpINS reactivity on response time interference, $F(1, 35) = 4.53$, $p < 0.05$, $\eta_p^2 = .12$; Figure 3D). The relationship between conflict reactivity in the mpINS and IC-RT decreased in the CFS group, but in the comparison group this relationship increased.

We found fatigue severity significantly predicted neural reactivity of the left amygdala and mpINS, explaining 16% and 14% of the variance, respectively (Table 2). Adding anxiety and depressive variables, did not improve the fit of the model.

Exploratory whole-brain effects of I-C are provided in Table 3. In short, adolescent CFS patients showed decreased response to conflict in the insula, precentral gyrus, middle temporal gyrus, cingulate, middle occipital gyrus, middle frontal gyrus, thalamus, precuneus and cuneus.

## Discussion

The main findings of this study were that adolescents with CFS were less able to engage the left amygdala and left mid-posterior insula (mpINS) in response to conflict compared to the healthy comparison group. Reactivity of the amygdala was associated with accuracy interference during conflict. Conflict-related activity in the mpINS was related to response

time interference. There were no associations between brain activity and depressive and anxiety symptoms. The CFS group did not produce a negative affect bias.

Within CFS group, we found inefficient response time interference performance; however, this effect was not significantly different between-groups. This finding is in contrast to studies on adult depression and anxiety disorder (Etkin, Prater, Hoeft, Menon, & Schatzberg, 2010; Etkin & Schatzberg, 2011) and trauma-exposed youths, which do produce significant group differences on interference performances (Marusak, Etkin, et al., 2015; Marusak, Martin, Etkin, & Thomason, 2015). The response times for congruent and incongruent trials were not significantly different between groups. However, the absence of a response time conflict effect within patients indicates inefficient response time processing and suggests the emotional information in the stimuli either did not aid in cognitive processing on congruent trials and/or did not interfere in cognitive processing on incongruent trials, as observed in healthy adolescents. During the congruent condition, the competing word stimulus is understood to prime cognition, as there is no conflict in stimuli semantics. In the incongruent condition, the competing word stimulus is understood to interfere with cognition, as there is conflicting semantics in the stimuli. Both conditions represent a degree of cognitive inhibition of the overlearned prepotent response to read the word. Consequently, the absence of a response time conflict effect in adolescent CFS seems to be due to inefficient processing of the word-distractor stimuli, which should either facilitate or interfere with cognition, accordingly.

Decreases in neural reactivity to conflict in the left amygdala and left mpINS indicate deficits in cognitive and affective functioning of the salience network (SN) (Marusak, Etkin, et al., 2015; Menon, 2015). Perceptive sensory areas send information to amygdala and FIC that is further relayed to the mpINS (Cauda, Geminiani, & Vercelli, 2014). The lateralized left amygdala and mpINS effects found in our study might be related to the different roles of

hemisphere function in emotional processing. In a systematic review, researchers found predominate left amygdala activation across *f*MRI studies (Baas, Aleman, & Kahn, 2004). Their study supports the theoretical view of functional brain asymmetry in the local and global processing of information. Local processing of the left hemisphere is relatively biased towards the processing fine-grained details of a stimulus or scene; whereas, global processing of the right hemisphere is relatively biased towards the processing of holistic aspects of a stimulus or scene (Hugdahl & Davidson, 2004). The decrease in left amygdala reactivity of adolescent CFS patients could suggest inefficient local functioning in the detection of salient stimuli cues that might influence further cognitive processing to the left mpINS.

Current SN theory suggests salience filtering occurs in the SN initiating cognitive control signaling that influence how stimuli are processed (Menon, 2015). As such, neural activity in regions responsible for detecting conflict should reflect the amount of behavioral interference, resulting in higher activity in those regions. Even though the behavioral interference measures did not reveal between-groups effects, we found the relationship between group differences and interference measures varied as a function of conflict-related neural activity in the amygdala and insula, which better illustrates group differences in brain behavior relations.

The negative association between accuracy interference and conflict-related reactivity in the left amygdala, and the lack of association between response time interference and conflict-related reactivity in the mpINS in CFS patients are indicators of inefficient salience detection and filtering function of the SN. In a previous study on childhood CFS, increasing poor task performance in patients resulted in a less efficient use of neural resources in frontal regions, where greater mental effort afforded costly energy requirements (Mizuno et al., 2015). Exertion intolerance experienced by CFS patients might be a consequence of impoverished cognitive control signaling from SN regions that results in inefficient use of neural resources and energy consumption, a vicious cycle that increases fatigue.

Adolescent CFS group and comparison group did not differ on demographic factors, allowing us to compare effects of fatigue in groups that had similar sociodemographic backgrounds. Thus, the observed neural changes may represent specific fatigue related alterations, as symptoms of depression and anxiety did not influence the relationship between fatigue severity and neural function. Additionally, negative affect bias to faces expressing negative emotion, a surrogate maker in anxiety and depression disorders (Bar-Haim et al., 2007; Gotlib et al., 2004) and a developmental effect seemingly amplified during childhood (Augusti, Torheim, & Melinder, 2014), was not observed in the CFS group of this study. Within the adolescent CFS patients, psychiatric disorders were ruled out prior to inclusion in this study (Sulheim et al., 2014). Even though they reported significantly more anxiety and depressive symptoms than the healthy comparison group, they did not produce this bias effect. In fact they were less accurate on both happy conditions suggesting trials that should be relatively easy did not help cognition. The lack of neural associations with depressive and anxiety symptoms and the absence of a negative affect bias further supports CFS as an important clinical entity (Lamers, Hickie, & Merikangas, 2013), where the relationship between prolonged fatigue and neural dysfunction potentially threatens normal development in adolescents.

The decreased neural reactivity to conflict in adolescent CFS patients is not clear. Following the *Sustained Arousal* model of disease mechanisms in CFS, a maladaptive stress response is a central pathophysiological element (Wyller et al., 2009), eliciting autonomic and neuroendocrine alterations that parallel the pathophysiology of chronic PTSD (Pervanidou & Chrousos, 2010; Sulheim et al., 2014). Prolonged stress affects both the structure and function of the PFC and stress related alterations occur in regions mediating the highest levels of cognitive function (see McEwen and Morrison (2013) for review). Neuroimaging findings on trauma-exposed youths at-risk for stress-related psychopathology showed a similar abnormal

activity pattern in regions of the SN (Marusak, Etkin, et al., 2015). Even though SN dysfunction seems to parallel those that are stress-related, the neural responses were opposite in CFS adolescents.

A supplemental frame for the findings of this study might be alexithymia, which literally means - no words for emotions. Individuals suffering from alexithymia have difficulty in verbally describing their own emotions and are known to be comorbid with a number of psychiatric conditions (Taylor, Bagby, & Parker, 1999). Previous CFS research has reported alexithymic correlates in adolescents and adults (Sepede et al., 2011; van de Putte, Engelbert, Kuis, Kimpen, & Uiterwaal, 2007). Future research could focus on the connection between the clinical phenomena of alexithymia and the apparent "washing out of emotions" in adolescent CFS, suggested by the deficits in salience detection observed in this study.

***Strengths and limitations***

Using an adolescent patient population, it might be easier to identify real disease mechanisms as opposed to secondary phenomena associated with years of chronicity. As the findings of our study are only preliminary, future studies should confirm the results in a larger sample of patients. Furthermore, a causal relationship cannot be inferred in a cross-sectional design. We used liberal inclusion criteria, where not all patients adhere to the Fukuda-definition that is most widely accepted. Of the CFS patients allocated to this study, there were extenuating circumstances that reduced the number from 30 to 15 (i.e. not wanting to participate, having orthodontic treatment, and movement). So far as can be determined, there was no reason to suspect selection bias.

The emotional conflict task used in this study did not include a neutral condition, which would have helped to dissociate interference related to emotion in patients. Specifically, a neutral face condition would provide information to see if the lack of response time conflict

effect in patients was due to the inability to perceive emotion. The paradigm design did not allow us to measure conflict regulation. Conflict regulation effects provide information to dissociate neural regions active specifically in the regulation of emotional conflict. However, future research might consider using these aspects to further understand alterations in emotional conflict processing of adolescent CFS patients.

### *Conclusion*

Our data highlight behavioral and neural deviations in emotional conflict processing of adolescent CFS patients and deficits in these neural regions may contribute importantly to the maintenance CFS pathology. Our findings of amygdala and insula dysfunction demonstrates a disruption in cognitive and affective functioning of the salience network, a potential biomarker in developmental psychopathology (Uddin, Supekar, Ryali, & Menon, 2011). However, it is unknown whether impairments in salience detection during emotional conflict processing in adolescent CFS reflect a disorder-specific abnormality or a more general endophenotype of complex symptom disorders, such as chronic stress and pain. Nevertheless, the group differences suggest that the inability of patients to efficiently and effectively resolve emotional conflict is an important aspect of the pathophysiology of adolescent chronic fatigue syndrome and potentially of other chronic disorders. This nature of research warrants further investigation.

### Conflict of Interest Statement

None.


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**Author Contributions**

LW carried out data analyses and drafted the manuscript. TE and VBW conceptualized and contributed to the study design. DS and EF collected clinical data and contributed to the study design. AM and MØ contributed to the study design. All authors contributed to data interpretation and drafting of the manuscript.

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FIGURE 1. Emotional conflict task

Figure 1 shows a sample task time course illustrating the contrasts made to examine emotional conflict effect using incongruent and congruent trials. Participants were asked to respond to the emotional face and ignore the word.

TABLE 1. Demographic and clinical characteristics of adolescent Healthy Comparison Participants and Patients with Chronic Fatigue Syndrome in an fMRI Study

| Characteristic | Patients with Chronic Fatigue Syndrome (N=15) | | Healthy comparison group (N=24) | | p |
|---|---|---|---|---|---|
| | N | % | N | % | |
| Female | 14 | 93 | 16 | 67 | n.s. |
| [a]Fukuda criteria | 10 | 67 | | | |
| [b]NICE criteria | 12 | 80 | | | |
| | Mean | SD | Mean | SD | |
| Disease duration in months | 19.5 | 10.7 | | | |
| Age | 15.8 | 1.5 | 15.2 | 1.6 | n.s. |
| [c]BMI | 22.5 | 3.6 | 20.2 | 2.9 | n.s. |
| [d]WASI IQ | 107.9 | 13.3 | 115.3 | 17.1 | n.s. |
| Depression [e]MFQ | 15.9 | 8.4 | 5.6 | 6.9 | <0.001* |
| Spielberger State Anxiety Inventory | 20.3 | 4.8 | 16.0 | 3.5 | <0.01* |
| Chalder Fatigue Questionnaire | 19.7 | 6.5 | 9.0 | 3.6 | <0.001* |

[a]Participants fulfilling the Fukuda-definition of CFS (Fukuda et al., 1994)
[b]Participants fulfilling the National Institute for Health and Care Excellence (2007) definition of CFS
[c]Body Mass Index
[d]Wechlser Abbreviated Scale of Intelligence-estimated full IQ
[e]Mood and Feelings Questionnaire for Depression
*Indicates group comparison is significant at $p \leq 0.05$.
The $\chi^2$ test was used for sex; two-sample *t*-tests were used for continuous variables.
Not significant (n.s.)

FIGURE 2. Behavioral response interference to emotional conflict in adolescent patients with Chronic Fatigue Syndrome and Healthy Comparison participants

Figure 2 shows response time and accuracy interference scores reflecting the emotional conflict effect (I-C). **Panel A**, adolescent CFS patients did not show response time interference for incongruent relative to congruent trials (I-C). Higher values indicate a loss in performance. ** One-sample *t*-test, $p$ <0.001. **Panel B**, adolescent CFS patients show a similar loss of accuracy for incongruent relative to congruent trials (I-C). Negative values indicate a loss in performance. * One-sample *t*-test, $p$ <0.01. Error bars represent standard error.

TABLE 2. Linear regression: conflict-related activity in the left amygdala and mid-posterior insula associated with fatigue severity.

| **Left Amygdala** | **Model 1** | | | **Model 2** | | |
|---|---|---|---|---|---|---|
| | ***B*** | ***SE*** | ***ß*** | ***B*** | ***SE*** | ***ß*** |
| (constant) | 0.25 | 0.12 | | 0.46 | 0.25 | |
| Fatigue | -0.02 | 0.01 | -0.40** | -0.03 | 0.01 | -0.47** |
| Depression | | | | 0.01 | 0.01 | 0.28 |
| Anxiety | | | | -0.02 | 0.02 | -0.19 |
| $R^2$ | | 0.16 | | | 0.20 | |
| *F* | | 6.85** | | | 2.89* | |
| $\Delta R^2$ | | | | | 0.04 | |

| **Left mpINS** | **Model 1** | | | **Model 2** | | |
|---|---|---|---|---|---|---|
| | ***B*** | ***SE*** | ***ß*** | ***B*** | ***SE*** | ***ß*** |
| (constant) | 0.23 | 0.11 | | 0.36 | 0.23 | |
| Fatigue | -0.02 | 0.01 | -0.38* | -0.02 | 0.01 | -0.34 |
| Depression | | | | 0.00 | 0.01 | 0.03 |
| Anxiety | | | | -0.01 | 0.02 | -0.13 |
| $R^2$ | | 0.14 | | | 0.16 | |
| *F* | | 6.22* | | | 2.14 | |
| $\Delta R^2$ | | | | | 0.01 | |

**Note:** * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

TABLE 3. Group differences in Whole-Brain activity during conflict (Incongruent minus Congruent)

| Contrast | Brain Area | BA | Peak x | Peak y | Peak z | # of voxels | T-Score |
|---|---|---|---|---|---|---|---|
| *comparison > CFS* | | | | | | | |
| | R Parahippocampus | 36 | 30 | -40 | -8 | 17 | 4.35 |
| | L Insula | 13 | -39 | -7 | 7 | 223 | 4.23 |
| | R Parietal Precuneus | 7 | 15 | -76 | 40 | 192 | 4.15 |
| | R Lentiform Nucleus | | 21 | -10 | 4 | 14 | 3.83 |
| | L Mid Temporal gyrus | 37 | -48 | -67 | 4 | 65 | 3.75 |
| | R Insula | 13 | 39 | 5 | 16 | 12 | 3.65 |
| | R Precentral gyrus | 44 | 57 | 8 | 10 | 82 | 3.58 |
| | R Mid Occipital gyrus | 18 | 24 | -91 | -2 | 24 | 3.52 |
| | R Mid Frontal gyrus | 6 | 21 | -7 | 40 | 46 | 3.51 |
| | R Mid Occipital gyrus | 37 | 39 | -67 | 1 | 47 | 3.44 |
| | L Inferior Parietal | 40 | -42 | -46 | 55 | 26 | 3.40 |
| | R Putamen | | 27 | 17 | 4 | 55 | 3.39 |
| | R Superior Frontal gyrus | 6 | 15 | -1 | 64 | 26 | 3.38 |
| | L Thalamus | | -15 | -22 | 7 | 44 | 3.33 |
| | L Posterior Cingulate | 30 | -18 | -61 | 4 | 13 | 3.24 |
| | R Inferior Parietal | 40 | 45 | -37 | 52 | 17 | 3.22 |
| | R Cuneus | 17 | 18 | -76 | 10 | 26 | 3.21 |
| | L Precentral gyrus | 6 | -27 | -13 | 64 | 17 | 3.10 |
| | L Postcentral gyrus | 2 | -45 | -25 | 37 | 12 | 2.97 |
| | L Cingulate gyrus | 32 | -9 | 8 | 40 | 10 | 2.96 |
| *CFS > comparison* | | | | | | | |
| | no voxels survived at this threshold | | | | | | |

Exploratory whole-brain results provided at $p < 0.005$ uncorrected, cluster minimum = 10 voxels. Abbreviations: BA, Brodmann's Area; L, left; R, right. Coordinates are given in MNI convention.

FIGURE 3. Left Amygdala (AMY) and left mid-posterior insula (mpINS) responses to emotional conflict

Figure 3 shows decreased (A) left AMY and (C) left mpINS responses to emotional conflict in adolescent CFS patients compared to healthy comparison (HC) group. (B) The linear relationship between left AMY conflict-related activity and accuracy interference significantly differed in CFS patients than HC. (D) The linear relationship between left mpINS conflict-related activity and response time interference significantly differed in CFS patients than HC. Data used for plotting are extracted data from 8mm ROI spheres. *$p \leq 0.02$ two-sample *t*-test. Error bars represent standard error.