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Omega-3 polyunsaturated fatty acid levels and dysregulations in biological stress systems

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ABSTRACT

Background: Studies have shown that omega-3 (n-3) Polyunsaturated Fatty Acids (PUFAs), including docosahexaenoic acid (DHA), might have beneficial effects on somatic and mental health, potentially partly due to their mitigating effects on three major biological stress systems: the immune-inflammatory system, the hypothalamic-pituitary-adrenal-axis (HPA-axis) and the autonomic nervous system (ANS).
Objective: To examine the association between (cumulative measures of) markers of three biological stress systems and n-3 PUFA and DHA plasma levels.
Design: Plasma n-3 PUFA and DHA were measured using Nuclear Magnetic Resonance in 2724 participants from the Netherlands Study of Depression and Anxiety. Linear regression analyses (adjusted for sociodemographic, sampling, lifestyle and somatic disease variables) associated inflammation (C-reactive protein, interleukin-6, tumor necrosis factor alpha), HPA-axis (cortisol awakening response and evening cortisol) and ANS (heart rate, respiratory sinus arrhythmia and pre-ejection period) markers and cumulative indices within and across stress systems as independent variables with n-3 PUFA and DHA levels as dependent variables.
Results: Participants had a mean age of 41.8 (SD = 13.1) and 65.7% were female. Higher levels of all three inflammation markers (Beta = −.146 to −.073, all p-values < .001), evening cortisol (Beta = −.045, p = .033) and heart rate (Beta = −.080, p < 0.001) were significantly negatively associated with n-3 PUFA. Suggesting an exposure-response relationship, a higher number of markers indicative of inflammation and hyperactive HPA-axis (p < .001 and p = .003, respectively), but not of ANS dysregulation, was found in persons with lower n-3 PUFA levels. An exposure-response relationship was also found for having a higher number of different stress system dysregulations with lower n-3 PUFA levels (p < .001). For DHA, results were in line with those for n-3 PUFA, although with slightly smaller effect sizes.
Conclusions: Our study confirmed that having various (cumulative) indicators of dysregulation of three biological stress systems was significantly associated with lower n-3 PUFA and DHA plasma levels. If low n-3 PUFA levels are the cause of dysregulated stress systems, then n-3 PUFA supplementation might reduce biological stress and thereby improve somatic and mental health.

1. Introduction

Omega-3 (n-3) polyunsaturated fatty acids (PUFAs) consist of e.g. α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and are primarily found in fatty fish, along with some other seafood, and certain nuts and seeds (Simopoulos, 1999). N-3 PUFAs have anti-inflammatory properties and are suggested to positively influence brain functioning (Smith et al., 2011). Low levels of n-3 PUFAs are observed in patients with several somatic or mental disorders, and accordingly several intervention studies have found a beneficial effect of n-3 PUFA supplementation on, for example, cardiovascular disease, diabetes mellitus, obesity, inflammatory diseases, and neurological/neuropsychiatric disorders (Yashodhara et al., 2009), although this is not consistent in all studies (Kromhout et al., 2010; Rizos and Elisaf, 2017; Zhang et al., 2016).

It has been suggested that part of the beneficial effect of n-3 PUFAs on somatic and mental health is due to their mitigating effects on dysregulations of three major stress systems: the immune-inflammatory system, the hypothalamic-pituitary-adrenal-axis (HPA-axis) and the autonomic nervous system (ANS). These stress systems might in turn also affect n-3 PUFA levels. Dysregulations in all three systems have been implicated in the etiology of many somatic and mental disorders

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(Han et al., 2016; Incollingo Rodriguez et al., 2015; Pant et al., 2014; Penninx et al., 2013). Meta-analyses have shown that levels of inflammatory markers such as C-reactive protein (CRP), interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α) are increased in cardiovascular diseases (Gupta et al., 2017), obesity (Lasselin et al., 2014), rheumatoid arthritis (Shrivastava et al., 2015) and depression (Kiecolt-Glaser et al., 2015). Hyperactivity of the HPA-axis have been found in obesity (Incollingo Rodriguez et al., 2015) and higher cortisol levels at awakening have been observed in depressed patients (Vreeburg et al., 2009). ANS dysregulation, often indicated by increased heart rate (HR) and decreased Respiratory Sinus Arrhythmia (RSA) and Pre-Ejection Period (PEP) reflecting higher sympathetic and lower parasympathetic tone, has been associated with unfavourable metabolic health (Licht et al., 2010).

In addition to associations with mental and somatic diseases, the abovementioned biological stress systems have also been independently linked with n-3 PUFA levels. For example, in several studies high CRP, IL-6, and TNF-α plasma levels and chronic inflammatory diseases have been associated with low n-3 PUFAs plasma levels or intake (Ferrucci et al., 2006; Yates et al., 2014). Reviews on intervention studies have shown that n-3 PUFAs are effective in reducing inflammation in certain inflammatory diseases, such as rheumatoid arthritis (Yates et al., 2014), however there is no compelling evidence for other somatic diseases such as myocardial infarction (Hoogeveen et al., 2014). Regarding mental diseases, n-3 PUFA supplementation has shown to benefit depressed patients in some, but not all trials (Appleton et al., 2015).

With regard to the HPA-axis, were the first to report that elevated corticotrophin releasing hormone levels, which indirectly stimulates the synthesis of cortisol, were associated with low n-3 PUFA levels in humans (Hibbeln et al., 2004). N-3 PUFA deficiency has shown to induce HPA-axis hyperactivity in mice (Larrieu et al., 2014). Further research on the association between HPA-axis and n-3 PUFAs has mainly been focused on depressed patients. High evening salivary cortisol levels were significantly associated with low DHA plasma levels in depressed patients (Mocking et al., 2013). A decrease in cortisol after n-3 PUFA supplementation has been consistently observed (Hellhammer et al., 2012). A recent review suggests that the increased ratio of omega-6 (n-6) PUFA (generally seen as pro-inflammatory) to n-3 PUFAs might contribute to hyperactivity of the HPA-axis in depression through low-grade inflammation by means of pro-inflammatory eicosanoids and cytokines (Husted and Bouzinova, 2016). In turn, cortisol influences the mobilization (Brenner et al., 2001; Conner et al., 1996; Macfarlane et al., 2008), lipolysis (Brenner et al., 2001), oxidation (Flerov et al., 2003; Hibbeln and Salem, 1995) and synthesis (Brenner et al., 2001; Hibbeln and Salem, 1995) of fatty acids.

Regarding the ANS, only one study examined HR in relation to n-3 PUFA plasma levels and found an inverse association (Eide et al., 2016). A recent review described a positive association between HR variability and n-3 PUFA levels in nerve and heart tissue (Christensen, 2011). N-3 PUFA supplements have been able to reduce HR (Skulas-Ray et al., 2012), increase HR variability (Christensen, 2011) and increase PEP (Skulas-Ray et al., 2012). Both negative (Brouwer et al., 2006) and positive (Kromhout et al., 2011) effects of fish/n-3 PUFA supplementation on ventricular arrhythmias have been found, a disease of which the occurrence can be characterized by HR variability (Farrell et al., 1991).

It can be concluded that the strongest evidence for an association of the biological stress systems with n-3 PUFA and DHA is found for the markers of inflammation, followed by evening cortisol and HR, while little evidence is found for an association with cortisol at awakening, RSA and PEP. Most of the studies discussed had small sample sizes and focused on patients with one specific somatic or mental disorder (for example depression). Additionally, some used n-3 PUFA intake instead of plasma levels (which might be more prone to inaccuracy and recall bias, especially in patients with mood disorders) or studied an associated disease (ventricular arrhythmia instead of HR variability). Most studies only examined a limited number of markers of one stress system and did not focus on the three biological stress systems together. Although the interplay between the biological stress systems is well-known (Straub et al., 2005), the extent to which the cumulative dysregulations of these stress systems are associated with low n-3 PUFA levels is still uncertain.

Therefore, the present large-scaled study aims to establish the association of various indicators of inflammation (CRP, IL-6 and TNF-α), the HPA-axis (awakening and evening cortisol) and the ANS (HR, RSA and PEP) with n-3 PUFAs and DHA. We hypothesize that high inflammation markers, high HPA-axis activity, high sympathetic control (reflected in high HR and lower PEP) and low parasympathetic control (reflected in lower RSA) will be associated with low n-3 PUFA and DHA plasma levels. This study also aims to establish whether there is evidence of an exposure-response relationship between cumulative indicators of biological stress dysregulations and n-3 PUFA and DHA levels, both within and across all three systems.

2. Method and materials

2.1. Study sample

Participants were selected from the Netherlands Study of Depression and Anxiety (NESDA), a longitudinal observational cohort study (n = 2981) (Penninx et al., 2008). Between 2004 and 2007 in total 2981 participants aged between 18 and 65 years were recruited from the Dutch general population (19%), primary health care (54%) and specialized mental health care (27%). The research protocol was approved by ethics committees of participating universities. Participants provided written informed consent and blood samples (after instructions for overnight fast) and underwent a psychiatric interview. In total 69 participants (2.3%) were excluded due to in-complete blood samples, 162 participants (5.6%) due to corticosteroids use and 26 participants (0.9%) as they were pregnant and/or breastfeeding. The remaining participants (n = 2724) all had an available measurement on n-3 PUFA levels and at least one stress system marker. Further exclusions were made for analyses of specific stress markers due to subjects with missing data, with 8 (0.3%), 9 (0.3%) and 24 (0.9%) missing data points for CRP, IL-6 and TNF-α, respectively, 931 (34.2%), 931 (34.2%) and 772 (28.3%) missing data points for AUCg, AUCi and evening cortisol, respectively, and 117 (4.2%), 115 (4.2%) and 130 (4.8%) missing data points for HR, RSA and PEP, respectively.

2.2. N-3 PUFAs

A detailed description of the PUFA assessment can be found in a previous publication (Thesing et al., 2017). In short, fasting blood samples were collected in EDTA plasma tubes and stored at −80 °C. The iron chelator EDTA helps to inhibit iron-catalysed oxidation of PUFA's. N-3 PUFA and DHA have shown to be relatively stable when stored at −75 °C (Metherel et al., 2013). Blood samples were shipped in 2 batches for analysis (April and December 2014, further referred to as metabolic assessment wave 1 and 2, respectively). Among other metabolites, n-3 PUFA levels (including DHA) were quantified at 22 °C using a commercially available high-throughput proton Nuclear Magnetic Resonance (NMR) metabolomics platform (Nightingale Health Ltd., Helsinki, Finland) (Soininen et al., 2015). Fatty acids measured with this platform are esterified fatty acids stemming from the lipoprotein particles, representing fatty acids in the plasma that are bound within cholesteryl esters, triglycerides and phospholipids inside the lipoproteins particles. The DHA signal is quantified separately from a specific NMR signal unique to this molecule. The resolution of high throughput NMR does currently not allow sufficient resolution for robust, independent quantification of EPA due to spectral overlap. As described in a previous publication (Thesing et al., 2017), we primarily analysed relative n-3 PUFA measures (as percentage of total fatty acids yielding

mmol% values), and run secondary analyses using absolute values (mmol/l). Next to n-3 PUFA levels, levels of other fatty acids (e.g. those with opposite characteristics such as pro-inflammatory omega-6 (n-6) PUFAs) are important in the associations with these stress markers. Therefore, relative measures have found to be biologically more informative, reflecting PUFA levels in relation to overall fatty acids and better capture for instance the overall effect emerging from the pro-inflammatory/anti-inflammatory balance. This is supported by a recent study that found that presentation of fatty acids in either percentages or concentrations yields different results, particularly for those fatty acids with a stronger correlation with the total fatty acid level (Mocking et al., 2012). Finally, because of the pro-inflammatory characteristics of n-6 PUFA, we created a variable for the n-3:n-6 PUFA ratio, to examine association with the inflammation markers. Results of these sensitivity analyses are presented in a supplement.

2.3. Measurement of inflammation, HPA-axis and ANS

A detailed description of the measurement of the markers of inflammation, the HPA-axis and ANS can be found in the supplement. For inflammation, circulating plasma levels of CRP (N = 2716), IL-6 (N = 2715), and TNF-α (N = 2700) were assessed (Vogelzangs et al., 2012). For HPA-axis, both cortisol at awakening and evening cortisol were assessed in saliva. The area under the curve with respect to the ground (AUCg, N = 1793) and increase (AUCi, N = 1793) were calculated as measures of cortisol awakening response (CAR) with AUCg reflecting total 1-hour cortisol secretion after awakening, and AUCi the dynamics of the CAR (Licht et al., 2010). Evening cortisol levels (N = 1952) are considered to reflect basal cortisol secretion (Licht et al., 2010). Mixed models analyses were used in a previous study on the same HPA-axis data by Vreeburg et al. (2009) to create the cortisol awakening response (CAR) variable when some – but not all – morning cortisol levels were missing out of 4 saliva samples that were taken over time (at awakening, 30 min, 45 min and 60 min). The relatively large number of residual missing data points for HPA-axis markers was mainly due to participants who did not return saliva samples or collected them outside of a 5 min margin around the time protocol (n = 931 for cortisol at awakening and n = 772 for evening cortisol). Respondents on saliva collection did not differ from non-respondents in sex but were older (44.1 vs 38.2 years, respectively; $p < .001$), more educated (12.4 vs 11.6 years of education, respectively; $p < .001$), and less likely to be currently depressed (44.2% vs 57.4%, respectively; $p < .001$) (Vreeburg et al., 2009). Three ANS measures were derived: 1) heart rate (HR, N = 2607), reflecting the combined effect of sympathetic and parasympathetic activity; 2) respiratory sinus arrhythmia (RSA, N = 2609), reflecting parasympathetic activity; 3) pre-ejection period (PEP, N = 2595), reflecting sympathetic activity.

Consistently with a previous paper (Black et al., 2017), we decided a priori to create cumulative indices for each of the three stress systems (number of dysregulated markers, range 0–3). Therefore, we dichotomized the markers into 'dysregulated' (high-risk quartile) versus 'non-dysregulated' (other quartiles), as done previously. Only participants with complete data on all markers of the respective stress system were used (98.7%, 54.8% and 94.9% of the total sample of n = 2724 for inflammation, HPA-axis and ANS, respectively). Additionally, we calculated the sum of the number of stress systems (range 0–3) for which a subject had one or more markers falling within the high risk quartile. This cumulative index was only created for subjects who had complete data on all variables included in the count (42.8% of the sample of n = 2724).

2.4. Covariates and effect modifiers

A distinction was made between potential general confounders (the same for all biological stress markers) and potential specific confounders that differed for the biological stress markers, as done in a previous papers on the same data (Black et al., 2017). The general confounders were age (years), gender (male/female), blood sample collection area (Amsterdam, Leiden, or Groningen), metabolic assessment wave (1 or 2), fasting status at time of blood withdrawal (yes/no), and n-3 PUFA supplement use (yes/no). Use of n-3 PUFA supplements was derived from drug container inspection and was considered as this likely increases PUFA plasma levels.

For analyses on inflammatory markers, we additionally included systematic anti-inflammatory medication (Vogelzangs et al., 2012). In analyses with HPA-axis markers, awakening time (00:00–23:59), working on day of sampling (yes/no), season of sampling (October-February [less daylight] vs. March-September [more daylight]) were additionally included in the analysis (Vreeburg et al., 2009). Analyses with ANS markers, had additional adjustment for use of cardiac medication including beta-blocking agents and antihypertensive medication because these were determinants of ANS activity in this study (Houtveen et al., 2005). Analyses on RSA were additionally adjusted for respiratory rate. Analyses on PEP were additionally adjusted for mean arterial pressure ((systolic blood pressure + 2*diastolic blood pressure)/3) to account for potential between-subject differences in afterload (Houtveen et al., 2005).

As our study over-recruited persons with psychopathology, and as we previously found lower n-3 PUFA levels among persons with a current diagnosis of depression (Thesing et al., 2017), we briefly checked whether depression severity was a confounder or an effect modifier. Severity of depressive symptoms was based on the 30-item self-report Inventory of Depressive Symptomatology (IDS-SR30, ranging from 0 to 84) questionnaire, with higher scores indicating higher severity (Rush et al., 1996). Use of tricyclic antidepressants (TCA's), selective serotonin reuptake inhibitors (SSRI's) and use of other antidepressants was based on container inspection (yes/no).

2.5. Statistical analyses

Descriptive statistics were used to describe the study sample. For subsequent analyses, n-3 PUFA, DHA, CRP, IL-6 and TNF-α were $\log_e$ transformed due to skewed distributions. All dependent and independent variables were standardized to make results for the different stress markers more comparable. The associations between n-3 PUFA and DHA and each marker of the biological stress systems were examined in individual linear regression analyses with n-3 PUFA and DHA as dependent variables and the markers of the biological stress systems as independent variables. Markers were used both as continuous variables and as dichotomous variables (high risk quartile vs. rest). For all inflammation and HPA-axis markers and heart rate, the 4th quartile was defined as the high risk quartile. For PEP and RSA, the 1st quartile was defined as the high risk quartile, reflecting high sympathetic tone and low parasympathetic tone, respectively. To correct for multiple testing, the Benjamini-Hochberg False Discovery Rate method was used based on 36 tests (for 18 markers [both the continuous measures and high risk quartile measure] and 2 PUFA measures) (Benjamini and Hochberg, 1995).

Apart from individual stress markers, the accumulation of biological stressors might lead to even worse health outcomes. We therefore examined whether a priori made cumulative indices based on the number of dysregulated markers *within* one stress system were associated n-3 PUFA and DHA levels. Adjusted analyses of covariance (ANCOVAs) were performed per stress system to compare the mean n-3 PUFA and DHA levels (mmol%) between persons with gradually increasing number of markers in a high risk quartile (ordinal variables with 0, 1, 2 or 3 dysregulated markers). Multivariable linear regression analysis with the number of dysregulated markers for each stress system as independent variables and n-3 PUFA or DHA as dependent variables were performed to test whether stress systems were independently associated with n-3 PUFA and DHA.

In addition to testing cumulative indicators *within* a stress system, it

Table 1
Characteristics of the study sample.

		Mean (SD)/Median (IQR)/n %
Sociodemographic variables	N	
Age in years, mean (SD)	2724	41.8 (13.1)
Female, n (%)	2724	1789 (65.7)
Years of education, mean (SD)	2724	12.2 (3.3)
Blood sampling variables		
Fasting at time of blood withdrawal, n (%)	2724	2603 (95.6)
Blood sample collection area	2724	
Amsterdam, n (%)		1124 (41.3)
Leiden, n (%)		816 (30.0)
Groningen, n (%)		784 (28.8)
Metabolic assessment wave	2724	
First, n (%)		1482 (54.4)
Second, n (%)		1242 (45.6)
Specific confounders		
Use of systemic anti-inflammatory medication, n (%)	2724	108 (4.0)
Awakening time (00:00-23:59), mean (SD)	2724	7.46 (1.06)
Working on day of sampling, n (%)	2724	1951 (71.6)
Season of sampling	2724	
October–February (less daylight), n (%)		843 (30.9)
March–September (more daylight), n (%)		1881 (69.1)
Use of cardiac medication, n (%)	2724	326 (12.0)
Respiratory rate, mean (SD)	2609	17.6 (1.72)
Mean arterial pressure, median (IQR)	2720	97.8 (17.5)
Psychiatric disorder characteristics		
Current depressive disorder, n (%)	2724	779 (28.6)
IDS-SR_{30}, median (IQR)	2717	20.0 (22.0)
Use of antidepressants	2724	
No antidepressants, n (%)		2054 (75.4)
TCA, n (%)		72 (2.6)
SSRI, n (%)		455 (16.7)
Other antidepressants, n (%)		143 (5.2)
PUFAs		
n-3 PUFA (mmol%), median (IQR)	2724	3.17 (0.94)
DHA (mmol%), median (IQR)	2724	1.12 (0.50)
n-3 PUFA (mmol/l), median (IQR)	2724	0.36 (0.15)
DHA (mmol/l), median (IQR)	2724	0.13 (0.07)
Use of n-3 PUFA supplements, n (%)	2724	108 (4.0)
Inflammation		
C-reactive protein (mg/L), median (IQR)	2716	1.19 (2.40)
Interleukin-6 (pg/mL), median (IQR)	2715	0.74 (0.74)
Tumor Necrosis Factor α (pg/mL), median (IQR)	2700	0.80 (0.50)
Hypothalamic Pituitary Adrenal axis		
Area under the curve with respect to the ground (nmol/L/hr), mean (SD)	1793	18.9 (6.97)
Area under the curve with respect to the increase (nmol/L/hr), mean (SD)	1793	2.12 (6.22)
Evening cortisol (nmol/L), median (IQR)	1952	4.82 (3.24)
Autonomic Nervous System		
Heart Rate (bpm), mean (SD)	2607	68.7 (9.73)
Respiratory Sinus Arrhythmia (ms), median (IQR)	2609	39.5 (32.0)
Pre-injection period (ms), median (IQR)	2594	122.7 (25.3)

Note. Not-normally distributed variables are presented as medians and inter-quartile ranges. SD: Standard deviation. IQR: Interquartile Range. IDS-SR_{30}: 30-item self-report Inventory of Depressive Symptomatology. DHA: docosahexaenoic acid. n-3: omega-3. PUFAs: Polyunsaturated fatty acids. Bpm: beats per minute. Ms: milliseconds.

is also known that biological stress systems interplay and are correlated (Straub et al., 2005). The accumulation of dysregulations *across* systems might lead to even worse health outcomes. Therefore, the mean n-3 PUFA and DHA levels (mmol%) were calculated over each number of stress systems using adjusted ANCOVAs.

All analyses were repeated with absolute plasma levels (mmol/l) of n-3 PUFAs and DHA. We also examined potential confounding and effect modification by depression severity by additionally adding depression severity as a confounder and, in separate analyses, by entering stress system marker-by-depression severity interaction terms in our linear regression analyses. Finally, as some antidepressants have been associated with stress system markers and/or with n-3 PUFA and DHA previously within this sample (Licht et al., 2010; Thesing et al., 2017; Vogelzangs et al., 2012) we explored the role of antidepressant medication. Thus, analyses were repeated excluding antidepressant users. To check whether there is a potential impact of n-3 PUFA supplement use on the associations, a second sensitivity analysis was performed including only participants who didn't report n-3 PUFA use. All analyses were conducted using IBM SPSS statistics software, version 22 (IBM Corp., Armonk, NY, USA).

Table 2
Linear regression analysis showing the associations between biological stress measures with n-3 PUFA and DHA (mmol%; dependent variables).

	n-3 PUFA (mmol%)[a]			DHA (mmol%)[a]		
	Beta[b]	SE[c]	p-value	Beta[b]	SE[c]	p-value
Inflammation						
C-reactive protein						
Continuous[d]	−.146	.017	< .001*	−.031	.018	.089
Highest quartile	−.099	.018	< .001*	−.010	.018	.57
Interleukin-6						
Continuous[d]	−.086	.018	< .001*	−.052	.018	.004*
Highest quartile	−.110	.018	< .001*	−.086	.018	< .001*
Tumor Necrosis Factor α						
Continuous[d]	−.073	.018	< .001*	−.033	.018	.065
Highest quartile	−.090	.018	< .001*	−.048	.018	.007*
HPA-axis						
Area Under the Curve in respect to the ground						
Continuous	−.035	.022	.12	−.017	.022	.44
Highest quartile	−.034	.022	.12	−.022	.022	.31
Area Under the Curve in respect to the increase						
Continuous	−.016	.022	.47	−.008	.022	.70
Highest quartile	−.029	.022	.18	−.017	.022	.42
Evening cortisol						
Continuous	−.045	.021	.033	−.019	.021	.36
Highest quartile	−.077	.021	< .001*	−.054	.021	.011*
ANS						
Heart Rate						
Continuous	−.080	.018	< .001*	−.032	.019	.099
Highest quartile	−.068	.018	< .001*	−.036	.018	.054
Respiratory Sinus Arrhythmia						
Continuous	.013	.022	.54	.002	.022	.91
Lowest quartile	.005	.020	.81	.032	.021	.13
Pre-injection Period						
Continuous	.000	.018	.99	−.014	.019	.46
Lowest quartile	−.031	.019	.099	−.025	.019	.19

Note. Linear regression analyses were conducted with omega-3 Polyunsaturated Fatty Acids (n-3 PUFA) and docosahexaenoic acid (DHA) in mmol% as dependent variables. Main predictors were individual markers of stress systems (continuous measure and high risk quartile vs. rest). Adjustment for age, gender, blood sample collection area, metabolic assessment wave (1 or 2), fasting (yes/no), n-3 PUFA supplement use (yes/no) and specific confounders: use of systemic anti-inflammatory medication (yes/no) for inflammation, awakening time (00:00-23:59), working at day of sampling (yes/no), and season of sampling (light/dark) for HPA-axis, use of cardiac medication (yes/no), respiratory rate and mean arterial pressure for ANS activity. HPA-axis: Hypothalamic Pituitary Adrenal axis. ANS: Autonomic Nervous System.
[a]n-3 PUFA and DHA were transformed according to the natural log. [b]Standardized beta. [c]Standard error of standardized beta. [d]Inflammation markers were transformed according to the natural log. *Significant after correction for multiple testing according to the False Discovery Rate of Benjamini-Hochberg method based on 36 tests for n-3 PUFA and DHA combined.

3. Results

3.1. Patient characteristics

Table 1 shows the demographic characteristics of the total sample (N = 2724). Participants had a mean age of 41.8 (SD = 13.1) and 1789 (65.7%) were female. Markers within each stress system were intercorrelated (all p-values < .01, see Table S1). N-3 PUFA and DHA were also highly correlated (Spearman r = .85; p < .001). Significant correlations were found between almost all inflammatory markers and ANS makers (r ranging from -.19 through .20). Furthermore, significant correlations were found between AUCg and RSA and between evening cortisol and both RSA and CRP (Table S1).

3.2. Inflammation and PUFAs

After adjustment for confounders and after correction for multiple testing, high CRP (Beta = -.146, SE = .017, p < .001), high IL-6 (Beta = -.086; SE = .018; p < .001) and high TNF-α (Beta = -.073; SE = .018; p < .001) were significantly associated with low n-3 PUFA levels (see Table 2). High IL-6 (Beta = -.052; SE = .018; p = .004) and high TNF-α (only the high risk quartile: Beta = -.048; SE = .018; p = .007) were also significantly associated with low DHA. CRP (Beta = -.058, SE = .018, p = .001), IL-6 (only the high risk quartile, Beta = -.045, SE = .018, p = .011) and TNF- α (Beta = -.073, SE = .018, p < .001) were all significantly associated with the n-3:n-6 PUFA ratio (Table S5).

3.3. HPA-axis and PUFAs

High evening cortisol (only the high risk quartile; Beta = -.077; SE = .021; p < .001) was associated with low n-3 PUFA levels (Table 2). High evening cortisol (only the high risk quartile; Beta = -.054; SE = .021; p = .011) was also significantly associated with low DHA. For AUCg and AUCi, as expected, negative associations were found with n-3 PUFA and DHA, however all were non-significant.

3.4. ANS activity and PUFAs

Table 2 shows that high HR (Beta = -.080; SE = .018; p < .001) was significantly associated with low n-3 PUFA levels, while for DHA a negative and almost significant association was found. For RSA and PEP, as expected, positive associations were found with n-3 PUFA and DHA, however all were non-significant.

3.5. Cumulative index within the biological stress systems and PUFAs

In ANCOVAs exploring the relationship between the cumulative indices of each of the three systems (0–3 markers) with n-3 PUFA and DHA, a higher number of inflammation markers in the high risk quartile was found to be associated with lower n-3 PUFA and DHA levels (p for linear trend < .001 and < .001, Fig. 1A and D). The group with three high risk quartile inflammatory markers had significantly lower n-3 PUFA levels compared to the groups with no (Cohen's d = .60; p < .001) and one (Cohen's d = .34; p = .002) high risk quartile inflammatory markers. For DHA, the group with three high risk quartile inflammatory markers had significantly lower DHA levels compared to the groups with no (Cohen's d = .36; p = .001) or one (Cohen's d = .24; p = .044) high risk quartile inflammatory markers.

A high number of HPA-axis markers in the high risk quartile was only significantly associated with lower n-3 PUFA levels while not with lower DHA levels (p for linear trend .006 and .060, Fig. 1B and E). The group with three high risk quartile HPA-axis markers had significantly lower n-3 PUFA levels than the group with no markers (Cohen's d = .35; p = .012).

A high number of ANS markers in the high risk quartile was only significantly associated with lower n-3 PUFA levels while not with lower DHA levels (p for linear trend .024 and .285, Fig. 1C and F). The groups with three high risk quartile ANS markers had significantly lower n-3 PUFA levels than the groups with no, one or two markers (Cohen's d = 0.45; p = .016; Cohen's d = .60; p = .027; Cohen's d = .28; p = .032; respectively).

To confirm whether the association between a stress system and n-3 PUFAs was independent of the other stress systems, the cumulative indices of the three stress systems were entered *together* in one model. The cumulative indices for inflammation and HPA-axis were independently associated with n-3 PUFA and DHA, but the cumulative index of ANS was not (Table 3).

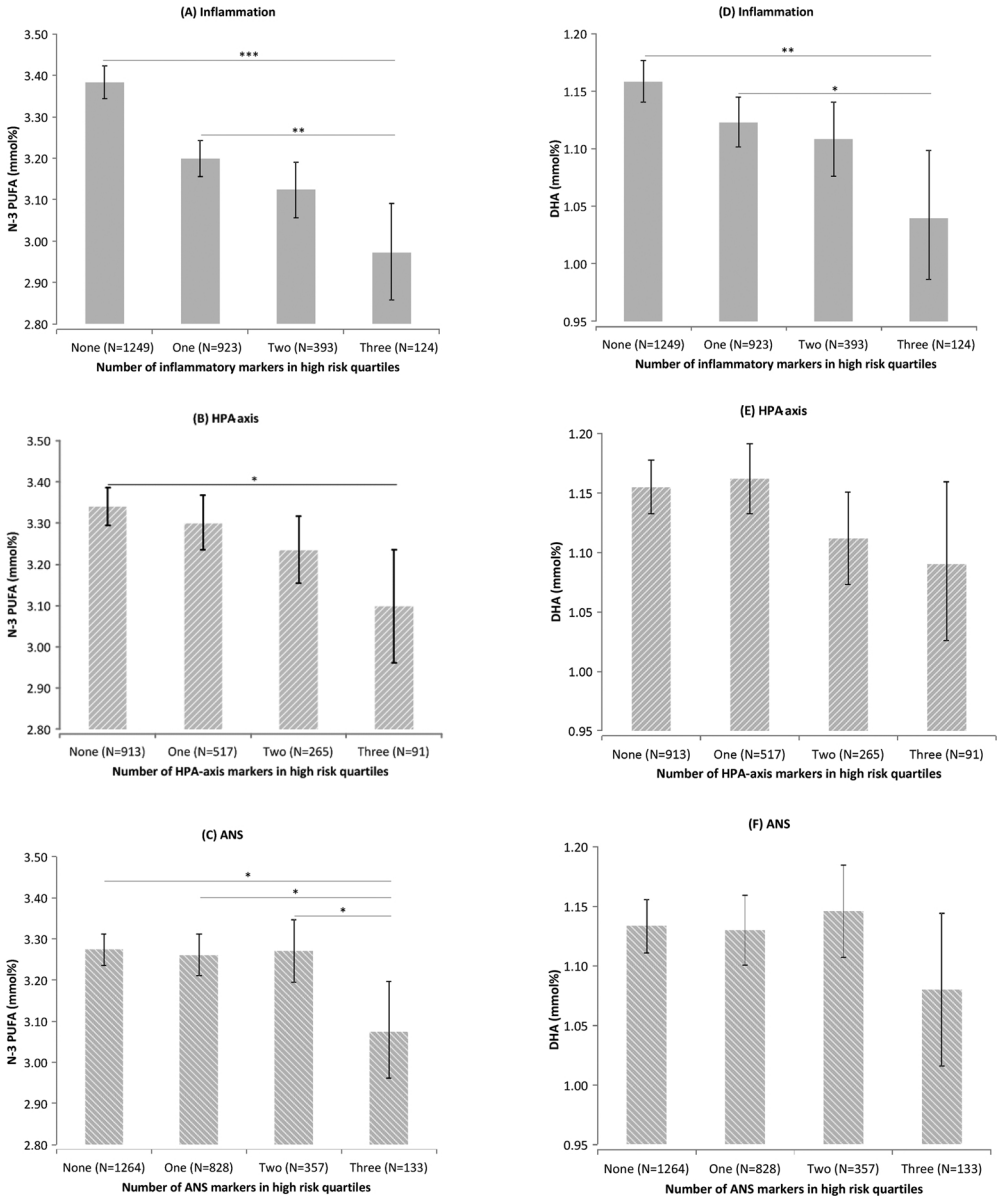

Fig. 1. Mean levels of plasma n-3 PUFA (A–C) and DHA (mmol%) (D–F) by number of inflammatory markers in high risk quartiles (A and D); number of HPA-axis markers in high risk quartiles (B and E); number of ANS markers in high risk quartiles (C and F). Fully adjusted ANCOVA's were performed with log transformed n-3 PUFA and DHA as outcomes. Means and error bars are back-transformed. HPA-axis: hypothalamic pituitary adrenal axis ANS: autonomic nervous system; N: number.

3.6. Total cumulative index across dysregulated biological stress systems and PUFAs

In analyses exploring the relationships between the cumulative measure of all three systems and n-3 PUFA and DHA, an increasing number of dysregulated stress systems (with at least one markers within the high risk quartile) was associated with lower n-3 PUFA levels (p for linear trend < .001), while for DHA no significant association was found (p for linear trend .199; Fig. 2). The group with dysregulation in all three stress systems had significantly lower n-3 PUFA levels than the groups with dysregulation in no, one or two systems (Cohen's d = .12; p < .001; Cohen's d = .21, p = .001; Cohen's d = .41, p = .043, respectively).

3.7. Sensitivity analysis

When repeating analyses using absolute levels as opposed to relative levels of n-3 PUFA and DHA, significant associations were found in the same direction, for IL-6 and TNF-a with n-3 PUFA and DHA (Table S2). In contrast to our findings using relative levels, significant associations were found for low CRP, low HR and high RSA with low DHA and for high RSA with low absolute levels of n-3 PUFA.

We examined whether found associations were influenced by depression severity, by adding depression severity to the model as an additional confounder and also by testing stress system marker-by-depression severity interaction terms between the 18 investigated continuous stress system markers and both n-3 PUFA and DHA. When adjusting for depression severity, the same significant associations were found. Subsequently, among the 18 stress system marker-by-depression severity interaction terms tested, only 1 (for AUCi in the association with n-3 PUFA) was significant after adjustment for multiple testing. This suggests that depression is unlikely to have influenced associations of biological stress markers with n-3 PUFAs and DHA levels. Sensitivity analyses in subsamples excluding antidepressant users (n = 2054) and users of n-3 PUFA (n = 2620) gave similar results (Tables S3 and Table S4, respectively), suggesting that antidepressant use or n-3 PUFA use do

Table 3
Linear regression analysis with the three stress systems independently and together as independent variables and n-3 PUFA and DHA levels as the dependent variables.

	Model 1						Model 2					
	n-3 PUFA[a]			DHA[a]			n-3 PUFA[a]			DHA[a]		
	Beta[b]	SE[c]	p-value	Beta[b]	SE[c]	p-value	Beta[b]	SE[c]	p-value	Beta[b]	SE[c]	p-value
# of inflammation markers	−.152	.018	< .001	−.074	.018	< .001	−.149	.023	< .001	−.077	.023	.001
# of HPA-axis markers	−.070	.022	.001	−.044	.022	.043	−.066	.022	.003	−.045	.023	.045
# of ANS markers	−.038	.019	.053	−.015	.020	.468	−.027	.023	.245	−.006	.024	.803

Note. HPA: Hypothalamic–pituitary–adrenal axis. ANS: Autonomic Nervous System. N-3 PUFA: omega-3 polyunsaturated fatty acids. DHA: docosahexaenoic acid. Model 1: separate linear regression analysis per stress system. Model 2: entering the stress systems together in the linear regression analysis. Both models were adjusted for age, gender, blood sample collection area, metabolic assessment wave (1 or 2), fasting (yes/no), n-3 PUFA supplement use (yes/no) and specific confounders: use of systemic anti-inflammatory medication (yes/no) for inflammation, awakening time (00:00–23:59), working at day of sampling (yes/no), and season of sampling (light/dark) for HPA-axis, use of cardiac medication (yes/no), respiratory rate and mean arterial pressure for ANS activity.
[a]n-3 PUFA and DHA were transformed according to the natural log. [b]Standardized beta. [c]Standard error of standardized beta.

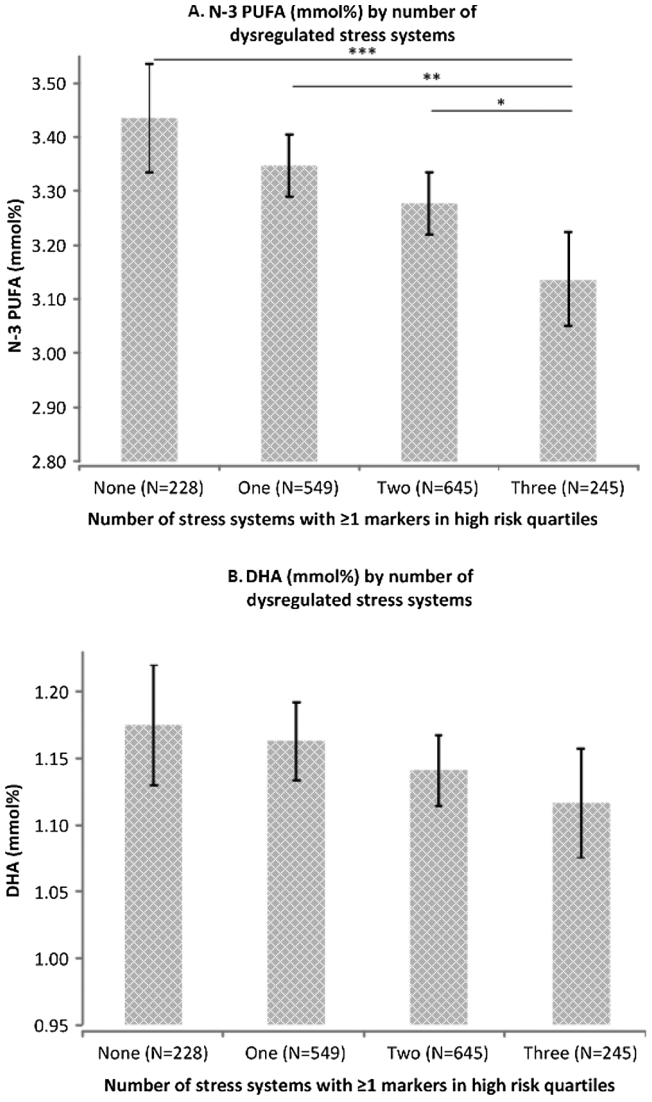

Fig. 2. Mean (a) n-3 PUFA and (b) DHA (mmol%) by number of stress systems with ≥1 marker in high quartiles. ANCOVA's were performed with log transformed n-3 PUFA and DHA (mmol%). Mean values and error bars are back-transformed. Analyses were adjusted as model 2.

not influence associations between biological stress markers and n-3 PUFA and DHA.

4. Discussion

The present large-scaled study examined associations of multiple markers of three major biological stress systems (inflammation, HPA-axis, and ANS) with n-3 PUFA plasma levels. Overall, it can be concluded that exposure-response relationships were found between on the one hand having a higher number of markers indicative of inflammation and having a higher number of markers indicative of a hyperactive HPA-axis and on the other hand low n-3 PUFA plasma levels. For the number of dysregulated ANS markers, relationships with n-3 PUFAs were found only with the highest risk group. Additionally, an exposure-response relationship was found between having a higher number of dysregulated stress systems and low n-3 PUFA plasma levels. For DHA, results were overall in line with those for n-3 PUFA, although with slightly smaller effect sizes.

Findings were consistent across individual inflammation markers, with higher levels significantly associated with low n-3 PUFA plasma levels, which is in line with earlier research (Ferrucci et al., 2006). Additionally, having multiple dysregulated inflammation markers was associated with lower levels of n-3 PUFA. While CRP, IL-6 and TNF-α are mainly seen as pro-inflammatory, n-3 PUFAs are seen as anti-inflammatory (Li et al., 2014). One of the mechanisms that might underlie the lowering effect of n-3 PUFA on CRP, IL-6 and TNF-α involves both nuclear factor kappa B (NF-κB), peroxisome proliferator agonist receptor (PPAR) and oxidative stress (Li et al., 2014). N-3 PUFAs is a natural ligand of PPAR, which inhibits the activation of NF-κB, that initiates genes encoding for TNF-α and IL-6. In turn, TNF-α leads to phosphorylation of IκBa, an inhibitor of NF-κB. Because high oxidative stress can lead to the activation of NF-κB, the anti-oxidative effect of n-3 PUFAs may also help explain its lowering effect on TNF-α, IL-6 and consequently on CRP production (Li et al., 2014). Furthermore, n-3 PUFAs are well known for inhibiting the production of pro-inflammatory cytokines such as IL-6 and TNF-α, via the reduction of the production of pro-inflammatory eicosanoids such as thromboxanes, some prostaglandins and leukotrienes (Calder, 2006). Although not yet investigated, pro-inflammatory cytokines are in turn hypothesized to affect the metabolic pathway (i.e., the fatty acid desaturase FADS gene cluster (Park et al., 2016)) of n-3 PUFA or the rate of beta-oxidation of n-3 PUFA. The negative associations between the inflammatory markers and the n-3:n-6 PUFA ratio are in line with the significant negative associations between the inflammatory markers and n-3 PUFA. This is expected as n-3 PUFA and the n-3:n-6 PUFA ratio are highly correlated (Spearmans rho = .90, p < .001)

For the HPA-axis, evidence was found for an exposure-response

relationship between having a high number of markers indicative of a hyperactive HPA-axis and low n-3 PUFA plasma levels. High evening cortisol levels were significantly associated with low n-3 PUFA plasma levels, which is in line with a previous study (Mocking et al., 2013). Negative but non-significant associations were found between cortisol at awakening and n-3 PUFA plasma levels. Additionally, having multiple dysregulated HPA-axis markers was associated with lower levels of n-3 PUFA. Evening cortisol levels are considered to reflect basal cortisol secretion: the ability of the HPA-axis to return to lower levels of cortisol towards the end of the day after the morning peak levels in the cortisol awakening response (Licht et al., 2010), and may better capture HPA dysregulations than morning cortisol levels do (Mocking et al., 2013). Cortisol may affect the mobilization, lipolysis, oxidation and synthesis of fatty acids (Mocking et al., 2013). In turn, fatty acid alterations affect the activation and feedback mechanism of the HPA-axis by influencing glucocorticoid receptor functioning, the transport of cortisol across the blood-brain barrier and pro- and anti-inflammatory eicosanoids which influence corticotrophin releasing hormone secretion (Mocking et al., 2013).

Analyses of the ANS found a non-linear association with n-3 PUFA. High HR was significantly associated with low n-3 PUFA levels, which is consistent with a previous study (Eide et al., 2016). HR is a measure of both parasympathetic and sympathetic control (Licht et al., 2010). Studies have shown that n-3 PUFAs reduce membrane electrical excitability of the cardiac myocyte by lowering its resting membrane potential and the duration of the refractory period through inhibition of ion channels, resulting in lower HR (Kang, 2012). The non-significant association of RSA with n-3 PUFA is not in line with a previous review which found a positive association between n-3 PUFA levels in nerve and heart tissue and HR variability, although in these studies HR variability was expressed by other measures than RSA (Christensen, 2011). This is the first study to investigate associations between PEP and n-3 PUFA, and therefore our lack of significant association can not be corroborated.

As expected, associations were largely consistent between absolute and relative measures of n-3 PUFAs, and between n-3 PUFAs and DHA, for all stress systems, although some inconsistencies have been noted. For example, high DHA levels (mmol/l) were associated with high CRP, which is contrary to some suggestions that n-3 PUFAs are anti-inflammatory agents (Smith et al., 2011). This finding may be a chance finding, yet, this is in line with a recent study that found that relative and absolute PUFA measures may yield different results, particularly for those fatty acids with a stronger correlation with the total fatty acid level (Mocking et al., 2012). The different fatty acids all have a variety of properties, for example some have anti-inflammatory properties (i.e. n-3 PUFA and DHA) while other have pro-inflammatory properties (i.e. n-6 PUFA). Therefore high absolute DHA levels in a person may be found together with high levels of inflammation markers such as CRP, because high absolute DHA levels can co-exist next to even higher absolute n-6 PUFA levels, which results in an overall inflammatory state. This needs to be examined further.

Overall, our findings indicate that – within a stress system – both the dysregulation of individual markers as the accumulation of dysregulated markers within one stress system is related to lower n-3 PUFAs. For the number of inflammation and HPA-axis markers, these associations with n-3 PUFA persisted while adjusting for each other and the number of dysregulated ANS markers. Additionally, the small but significant intercorrelations between the stress systems indicate that the stress systems do not reflect similar constructs. So, with respect to the accumulation of dysregulations across stress systems, having more than one stress system with at least one dysregulated marker was associated with lower n-3 PUFA levels. Hence, both the dysregulation of individual stress systems and the accumulation of dysregulated stress systems seem important. This points to an interplay between biological stress-systems which is independent from their individual effects, as found by Straub et al. (2005).

The complex relationship between fatty acids and biological stress most likely resembles a vicious circle. Low n-3 PUFA levels may have subtle effects on diverse pathways leading to a pro-inflammatory state and a chronically heightened stress response. The physiological responses to biological stress might in turn be a lowering of circulating n-3 PUFAs, directly, or indirectly via a lowering of n-3 PUFA intake. This weakens the idea of low n-3 PUFA plasma levels as merely a marker of overall bad health (innocent bystander) or the conviction that they tag potentially pathological stress processes (even if they are not yet overtly clinical). In addition, part of the alterations in fatty acids and biological stress may be adaptive, i.e. alterations instead of dysregulations (Assies et al., 2014). Meta-analyses for most diseases in which inflammation, the HPA-axis or the ANS have a role do not support the use of n-3 PUFA supplementation in their prevention and/or treatment. Nevertheless, the results from this study may point to new pathways that could be modified in order to improve health. Take for example depression. Interesting is the finding of Rapaport et al. (2016) in patients with MDD, showing that it is not necessarily low n-3 PUFA baseline levels that predict response to supplementation (i.e. correction of an n-3 PUFA deficit), but baseline inflammatory markers that predict response to supplementation. Most depression reduction response was seen in EPA supplemented patients with high inflammatory markers (i.e. the anti-inflammatory effects of EPA correcting an inflammatory state in the depressed patients) compared to EPA supplementation in depressed patients with low inflammatory markers. This fits with our findings as relationships with total n-3 PUFA levels (including EPA next to DHA) were stronger than with DHA levels.

Besides DHA, no other constituent of n-3 PUFA was measured, including EPA. We measured N-3 PUFA in plasma, and N-3 PUFA levels in serum, plasma, or erythrocyte membrane phospholipids can vary substantially based on an individual's meals in the past weeks or months and therefore reflect medium-term dietary intake, while fatty acid levels in adipose tissue reflect long-term intake (Thiebaut et al., 2009). In the present study, participants with a diagnosis of depressive/anxiety disorder were overrepresented, which could influence the generalizability of the results to the general population. However, there was no effect modification or confounding by depression severity so depression is unlikely to have influenced associations of biological stress markers with n-3 PUFAs and DHA levels. Results of analyses with HPA-axis data and the resulting cumulative measures had missing data points due to non-response. This could potentially bias the observed association if the missings were selective. Correction for multiple testing has been applied based the total number of tests in this paper. However, we have published on PUFAs before using the same dataset and one could suggest to take the number of tests in these papers also into account in the overall type 1 error rate. Strengths of our study are the inclusion of multiple markers of biological stress system dysregulations for which the individual and joint associations with n-3 PUFA were examined, and the large range of covariates that were taken into account.

5. Conclusions

In summary, it can be concluded that greater dysregulation of (the markers of) the three biological stress systems is associated with low n-3 PUFA and, to a lesser extent, with low DHA plasma levels. This implies that n-3 PUFAs are associated with an interplay of these stress systems, on top of the individual stress systems and their markers. This relationship is most likely bidirectional. Both low N-3 PUFA levels and dysregulation of the three stress systems have been associated with many somatic and mental disorders (Penninx et al., 2013). Intervention studies with n-3 PUFA supplementation can provide insight whether n-3 PUFAs are able to reduce biological stress. If low n-3 PUFA levels are the cause of dysregulated stress systems, then n-3 PUFA supplementation may be useful in the treatment of many somatic and mental disorders via their mitigating effect on the three major biological stress systems.

Conflict of interest

BWJH Penninx has received (non-related) research funding from Janssen Research and Boehringer Ingelheim. None of the other authors have any conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.07.002.

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