Immune-to-brain communication in functional somatic symptoms

Methodological considerations and preliminary evidence

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Cover design by Joyce Vermeer

ISBN 9789039360163
Printed by Gildeprint Drukkerijen, The Netherlands

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Immuun-breincommunicatie in somatisch onvoldoende verklaarde lichamelijk klachten

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan,
ingevolge het besluit van het college voor promoties in het openbaar te verdedigen
op vrijdag 18 oktober 2013 des middags te 12.45 uur

door

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geboren op 30 oktober 1979 te Ede

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“The mind cannot win over matter, for once the mind is asked to do too much, it quickly shows itself to be matter as well.”

Paul Auster, *Moon Palace*

Contents

Chapter 1

General Introduction

Introduction

When a person presents with physical symptoms that cannot (fully) be explained by organic pathology, these symptoms will be labelled “medically unexplained” or “functional” or, as used in the Dutch multidisciplinary directive[1]: “somatically insufficiently explained physical symptoms”. In this thesis, the term “functional somatic” (FS) will be used to refer to such symptoms. The prevalence of FS symptoms in Western society is high with 2.5 - 15.3% of patients in primary care and up to 66% of patients in secondary care presenting with such symptoms[2,3]. Most common symptoms are fatigue or pain-related[2,4]. FS symptoms are more common in women and are usually associated with a younger age[3,5-7].

Often, more than one symptom is present and certain constellations of symptoms give way to a diagnosis of a specific FS syndrome like, for example, chronic fatigue syndrome, fibromyalgia, or irritable bowel syndrome, with specific diagnostic criteria for each syndrome[8-10]. The Diagnostic and Statistical Manual of Mental Disorders version IV (DSM-IV-TR) offers classifications for severe and chronic FS symptom presentation, such as somatoform disorder, somatisation disorder, and pain disorder[11]. Both the FS syndrome diagnoses and the DSM-IV-TR classifications require a presentation of multiple somatic symptoms that cannot be explained by a medical condition. Of note, in the recently introduced DSM-V, the somatoform classifications are replaced by a general classification of somatic symptom disorder in which the requirement that the somatic symptoms must be medically unexplained is omitted, thereby eliminating a pure FS symptom classification from the DSM[12].

Criteria for participant-inclusion in FS symptom research is often based on syndrome and DSM-diagnoses, inherently regarding patients with separate diagnoses as separate clinical groups. However, the use of diagnostic labels based on presentation of specific symptoms has been disputed[13] and might unnecessarily limit generalizability of study results to other FS symptom patients.

A role for immune-to-brain communication in FS symptoms

An immunological perspective on FS symptoms was put forward based on the resemblance between common FS symptoms and the changes in behavior that occur in response to activation of the innate immune system[14]. Activation of the innate immune system leads to the peripheral release of pro-inflammatory cytokines such as interleukin(IL)-1β, IL-6, and tumor necrosis factor(TNF)-α. These peripheral cytokines act

on the brain through several pathways, leading to the expression of pro-inflammatory cytokines in the brain (central cytokines). Expression of central cytokines is associated with changes in behavior and mood, together coined "sickness behavior"[15,16]. Sickness behaviors are considered part of the body's strategy to fight the infection as they seem to promote fever, preventing heat loss and loss of energy, and by discouraging behavior that could lead to exposure to new infections or damage[15,17].

Table 1 provides with an overview of commonly found changes in behavior and mood in animals and humans during (experimentally induced) inflammation. As this table shows, FS symptoms indeed seem to resemble sickness behavior, with pain sensitivity and fatigue being among the core symptoms in both constructs and with both also including the presence of negative mood symptoms. It has to be noted however, that most knowledge on sickness behavior is still based on animal studies, where (objective) behavioral outcomes of inflammation are utilized. Human models of sickness behavior up till now include a limited range of possible sickness behavior-outcomes and mostly rely on (subjective) self-report. To increase insight in immune-to-brain communication in humans, objective measures of sickness behavior (such as increased pain sensitivity and impaired cognitive functioning) are necessary.

Table 1. Behavioral and mood outcomes (sickness behaviors) of generally used inflammatory models in animals and humans.

	Inflammatory models	Sickness behaviors
Animal studies[18-22]	Administration of lipopolysaccharide (LPS)[a] or pro-inflammatory agents (peripheral or central)	• Decreased food intake • Decreased exploration • Decreased sexual behavior • Increased anxious and depressive behavior • Increased pain sensitivity • Impaired cognitive functioning
Human studies [23-28]	Administration of LPS or *Salmonella typhi* vaccination; Treatment with high doses of pro-inflammatory agents[b]	• Increased fatigue • Increased depressive mood • Increased pain sensitivity • Reduced cognitive functioning

[a] Major constituent of the outer cell membrane of Gram-negative bacteria.

[b] High doses of IFN-γ and IL-2 are used in the treatment of certain types of cancer.

Sensitization of immune-to-brain communication

Animal models now also implicate the possibility of sensitization of immune-to-brain communication by exposure to prior (multiple) stressors. Specifically, animal studies showed that the occurrence of (immunological, psychosocial, or nociceptive) stressors could prime the inflammatory and sickness behavior-response to a stressor occurring later in time[29,30], and that the experience of multiple stressors within a restricted time frame led to an amplified inflammatory and behavioral response[31-33]. Evidence is also emerging from animal studies that individual characteristics related to stress coping can affect the sensitization effect of multiple challenges. This was, for example, illustrated by a study of Gibb et al.[34], in which the synergistic effects of a social stressor followed by an immunological stressor led to a more pronounced sickness behavior profile in mice from a relatively high stress reactive strain compared to a low stress reactive strain. At the moment, there are only a few pioneer studies pointing to the same mechanism in humans[35,36].

The possibility of sensitization of immune-to-brain communication seems of specific relevance for FS symptoms in humans as many FS symptom patients seem to fulfil the conditions for sensitization of immune-to-brain communication: Severe or chronic psychosocial stressors are related to the experience of severe or chronic FS symptoms[7,37-39] and the experience of infections has been indicated as a precipitating factor in the onset of FS symptoms in a subset of patients[40-42]. Furthermore, a history of multiple infections might predispose for the development of FS symptoms[43,44], although evidence for this is still limited and contradictory findings have also been reported[45]. Thus, research is needed that puts the theory of a sensitized immune-to-brain communication in FS symptoms to the test.

In designing such research, there are some considerations to take into account. First, sensitized immune-to-brain communication would only make sense if FS symptom patients have experienced more stressors in their past. As discussed above, the prevalence of psychosocial stressors is indeed higher, but evidence for a higher prevalence of immunological stressors is less strong and needs elaboration. Second, cross-sectional studies have not shown consistent differences in levels of inflammatory markers in FS symptom patients[46-48] (with the exception of patients with functional gastrointestinal symptoms, where evidence more consistently points to increased immunological activity[49]). It is possible that deviations in immunology become only visible after challenging the stress systems. Support for this can be found in reports on

exacerbated symptoms after strenuous activity and the described role of perceived stress in the perseverance of symptoms[50,51]. Third, sensitization of immune-to-brain communication might only be apparent at a central level, not showing deviations in peripheral inflammatory parameters (even after a stressor). Summarizing, the prevalence of immunological stressors in the past of FS symptom patients needs further elaboration as a condition for sensitized immune-to-brain communication to occur and research on the role of immune-to-brain communication in FS symptoms needs to incorporate active manipulation of immune-to-brain communication and to include both peripheral and central (or sickness behavioral measures that reflect central mechanisms) output measures.

Thesis outline

In the current thesis, some methodological issues are addressed that will come up when designing human studies for demonstrating sensitized immune-to-brain communication in FS symptom patients, with the aim of providing with the necessary tools to set up such research. Also, three studies will be presented that were set up for providing with preliminary evidence for sensitized immune-to-brain communication in humans.

Chapter 2 addresses the lumper-splitter debate on FS symptoms. While so-called "lumpers" regard FS symptom patients as one group, splitters argue that subgroups exist based on symptom presentation. This debate has implications for the inclusion of FS symptom patients in future (immune-to-brain communication) studies: should patients with specific symptoms or clusters of symptoms be included, or is it theoretically sound to include patients with FS symptoms regardless of the type of symptoms they present with? The latter seems more appropriate considering the wide range of symptoms that belong to the sickness behavior construct (in contrast to the symptom specificity that is inherent to syndrome labels), but it remains to be seen whether empirical support can be found for the lumpers approach. In this chapter, persons presenting with FS symptoms are clustered and solutions are screened for evidence in favour of either perspective.

Chapters 3 and 4 focus on possible objective measures of sickness behavior in humans. The assessment of sickness behavior in humans was relatively new when this thesis-project started and most studies have reported mainly on questionnaire data to describe changes in behavior and mood. More objective measures were needed, especially for studying persons with FS symptoms who are known to over-report.

Chapter 3 describes a study on the methodology of measuring pain sensitivity with algometry, leading to specific recommendations on its use. **Chapter 4** describes two studies that explore a possible objective outcome measure of cognitive fatigue, by combining cognitive performance and mental effort (using pupillometry) measures. Fatigue is a common FS symptom and also a frequently reported aspect of sickness behavior. Cognitive fatigue is regarded as a symptom of central fatigue (as opposed to physical fatigue, which can also represent peripheral fatigue) and might therefore be relevant for testing the immune-to-brain communication theory in FS symptoms.

In **Chapter 5,** a study is described that aimed to identify psychological and biological predictors of visceral pain sensitivity. Visceral pain sensitivity has been demonstrated in patients with functional gastrointestinal symptoms[52,53] and some preliminary evidence shows that it can be affected by experimentally induced inflammation[54]. If stress-related factors can be identified that predict visceral pain sensitivity, this would strengthen the idea of a sensitized immune-to-brain communication in these patients.

Chapter 6 presents a study on the prevalence of infections in persons with a somatoform disorder (characterized by multiple severe chronic FS symptoms) in the years prior to the psychiatric diagnosis as compared to controls. This study extends earlier findings on a history of increased infections in patients with FS syndromes.

In **Chapter 7**, an experimental placebo-controlled study focussing on sensitization of immune-to-brain communication in humans is described. The synergistic effects of psychosocial factors and an acute immunological stressor (i.e., a vaccination) on subsequent changes in inflammatory markers and pain sensitivity are reported.

Chapter 8 concludes with a summary of the previous chapters and a research proposal for future studies focussing on sensitisation of immune-to-brain communication in patients with FS symptoms.

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Chapter 2

"Functional somatic syndromes, one or many?": An answer by cluster analysis

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Journal of Psychosomatic Research, 2013; 74, 6-11

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Abstract

Objective: The aim of the present study was to address the lumper-splitter discussion on functional somatic syndromes by applying *k*-means cluster analyses on a heterogeneous sample of persons with unexplained somatic complaints. In favor of the lumper-side of the debate, clusters should differ only on the overall severity of the somatic complaints that were assessed. According to the splitters view, clusters should differ in symptom-specific patterns. ***Methods:*** Three-hundred ninety four subjects with functional somatic symptoms were clustered based on their scores on 47 somatic symptoms. Three cluster solutions (*k* = 2, 3, and 4 clusters) were compared on overall symptom severity, symptom patterns, and psychological distress. ***Results:*** Results showed that in all three solutions the clusters were defined by increasing total symptom scores and increasing psychological distress. Cluster-specific symptom patterns where evident only when more clusters (three or four) were allowed. The best fit index was found for a 2-cluster solution. ***Conclusion:*** The finding of symptom specific patterns in clusters which could not be differentiated on overall symptom severity is in favor of the splitters' view. The finding that all other clusters could be discriminated on overall symptom severity and that the 2-cluster solution had the best fit is in favor of the lumpers' view.

Introduction

When a person presents with somatic symptoms that cannot (fully) be explained by a known organic pathology, these symptoms will be labeled 'medically unexplained' or 'functional'. Often, more than one symptom is present and certain constellations of symptoms give way to a diagnosis of a specific functional somatic (FS) syndrome like for example chronic fatigue syndrome, fibromyalgia, or irritable bowel syndrome, with specific diagnostic criteria for each syndrome[1-3]. These FS syndromes have a high prevalence in our Western society[4,5].

The use of FS syndromes to diagnose persons with FS symptoms has been the topic of debate. The so-called splitters side of the debate defends the usefulness and even necessity to discriminate between syndromes as separate diagnostic categories[6,7]. Lumpers on the other hand argue that all of the syndromes represent one underlying common basic syndrome[8,9]. Arguments in favor of the latter position are as follows: a) the extensive overlap in core symptoms (e.g., fatigue, diffuse pain, general malaise); b) the fact that patients meeting criteria for one syndrome often meet criteria for other syndromes as well[8,9]; c) patients with different syndromes share non-symptom characteristics, like a history of stressful life events or a traumatic history[10,11]; and d) all syndromes share common psychiatric comorbidities (mainly anxiety disorders). Splitters argue that these arguments do not apply to all patients, and can thus not sufficiently explain the diversity and specificity of the syndromes. More recently, it has been suggested that both sides are true in that there is commonality as well as heterogeneity between (and within) FS syndromes in both onset-related factors and psychosocial and physiological patient characteristics[12].

Attempts have been made to solve the "splitters versus lumpers" debate on FS syndromes by statistical techniques such as principal components analysis that group FS symptoms to find specificities[13-15] or by latent class analyses to find communalities[16]. Some of the factor analytic studies have demonstrated multiple factor solutions with identifiable symptom groups per factor (e.g. gastrointestinal, musculoskeletal, cardiopulmonary)[13-16]. However, the symptom groups were found to differ between studies and the factors were inter-correlated[14,16], the most obvious reason being that patients often present symptoms from multiple factors. Therefore, grouping of symptoms across subjects may not be the appropriate statistical approach to solve the lumpers-splitters debate.

Of more relevance to the diagnosis of FS syndromes are techniques that try to categorize subjects in separate groups on the basis of the unique pattern of their symptoms. To date, only three studies have used this approach. Fink et al. used latent class analyses to identify groups in their sample[14]. These analyses yielded solutions with either two or three classes. In both results, classes could be distinguished by the *number* of symptoms and not by the *type* of symptoms. Gara et al. used hierarchical class analysis with a priori grouping of symptoms and found 11 patient clusters[17]. The clusters found in this study could be defined by “no symptom presentation”, “presentation of one group of symptoms”, “some groups of symptoms”, or “all groups of symptoms”. This partly confirms the findings by Fink et al. that persons can be clustered based on the number of symptoms (favoring a lumper’s position), but also points to the fact that for a number of patients the symptoms that are experienced belong to one symptom group only (favoring a splitter’s position). Finally, Kato et al. used latent class analysis of a sample of twins which yielded a five-cluster solution, with the clusters being different both in count of symptoms and in type of symptoms[18]; also supporting both the lumpers’ and the splitters’ side of the argument. Thus, these studies provide support for both sides of the argument, possibly implying that FS symptoms should be viewed from both a lumper’s and a splitter’s perspective.

Several methodological choices might have influenced the results in these studies. First, symptom presentation was dichotomized in most studies, thereby not taking into account symptom severity. This leads to giving an equal weight to vague side symptoms of a main symptom as to the main symptom itself. Second, the presence of physical symptoms was assessed over a relatively long retrospective period, either during the last two years[14] or during lifetime[17,18]. Symptom reports may therefore be strongly confounded by recall biases[19]. Third, predefined symptom groups were used in some studies[14,17], although the use of symptom groups is not strongly supported by factor analyses of the symptoms. Fourth, in two of the studies the data of healthy persons were combined with the data of patients in the same analyses[14,18]. As a result, the strongest differences within the sample are between the presence of *no* symptoms (i.e. the healthy persons) and the presence of *any* symptom (i.e. all the persons presenting with FS symptoms). This large difference between healthy subjects and patients may have masked a fine-grained cluster solution within patients. Based on these limitations it may be argued that clustering patients with FS symptoms needs further elaboration.

It is striking that the number of clusters found in the previous studies differs strongly, ranging from two to eleven. The number of clusters to search for is a matter of choice, and thus the subtlety of the solution (which depends on the number of clusters within the solution) is a consequence of this a-priori choice. Examining multiple cluster solutions within one sample could give some insight into the effects of the number of clusters on cluster structure.

In the current study, a sample of subjects with heterogeneous self-reported FS complaints was clustered on self-reported severity of 47 symptoms in the past seven days. The aim was to address the lumper-splitter discussion by examining cluster solutions on symptom severity and symptom patterns. In favor of the lumper-side of the debate, clusters should differ only on the overall severity of the complaints that were assessed. In contrast, according to the splitter-view, clusters should differ in symptom-specific patterns (i.e., syndromes). We chose to use a *k*-means clustering technique that allows for multiple cluster solutions as this technique allows for setting the number of clusters in a solution a priori and assigns each person into one cluster only (as opposed to other much used cluster techniques, such as latent class analysis). This way, it was possible to examine whether an a priori choice in the number of clusters in the solution would influence the explanation the cluster solution offers for the lumper-splitter debate. For further interpretation of the cluster solutions, the solutions were examined on between-cluster differences in total symptom scores, cluster-specific symptom patterns, and psychological distress. Psychological distress was included to incorporate the lumpers' position that the number of symptoms would be a function of the level of anxiety and depression.

Methods

Subjects

The source population consisted of subjects with heterogeneous FS complaints. Eligible participants were recruited through the internet, by placing links to the questionnaire on FS syndrome patient sites (i.e., for patients with chronic fatigue syndrome; fibromyalgia; irritable bowel syndrome; hyperventilation syndrome; and unexplained chronic pain). This way, it was ensured that only persons who consider themselves to have FS symptoms were exposed to the call. Only respondents who completed the survey were retained in the sample. The survey was started 653 times and completed 466 times. Fourteen

respondents completed the survey twice and for these persons the second response was deleted, resulting in a sample of 452 unique respondents. Two respondents were younger than 18 years of age at the time of responding and were deleted from the dataset. After exclusion of respondents who reported having used soft- or hard drugs in the last week (n = 5), having either an autoimmune disorder (n = 12), thyroid disorder (n = 29), or a disorder which leads to severe pain complaints (hernia, scoliosis, spondylosis, arthritis; n = 10), a sample of 394 respondents remained. Mean age at the time of responding was 48.4 years (range: 18 - 84) and the majority of the respondents was female (76.1%).

Procedure

The link on FS syndrome patient sites led to a homepage on which information regarding the study was posted. Persons who agreed to participate could click on a questionnaire-link on the homepage which automatically directed them to the information letter, entailing information about purpose and length of the questionnaire and storage of information. Informed consent was obtained for all respondents before entering the questionnaire. Respondents could give informed consent by checking the box below the information letter that said “I have read the information and agree to participate in this study”. The questionnaire was presented using NetQuestionnaires, version 6.5.

Measures

General descriptive information was obtained for gender, age, education, body length and body mass. Also, items were included concerning use of recreational drugs in the last week and presence of a chronic disease (reportedly diagnosed by a physician). It was also assessed whether a diagnosis of a FS syndrome was made in the past.

Somatic complaints were assessed with a 47-item symptom list specifically created for the current study (see appendix A). The list was primarily based on the Bodily Sensations Questionnaires[20] to which additional symptoms were added from several other somatic symptoms lists. Respondents could indicate for each symptom to what extent they had experienced this symptom in the last seven days on a five-point Likert scale (1 = not; 2 = a little; 3 = quite a bit; 4 = quite a lot; 5 = highly). The list included four gastrointestinal symptoms, six cardiac symptoms, five respiratory symptoms, six physical fatigue symptoms, six musculoskeletal symptoms, six cognitive symptoms, and fourteen ‘other’ symptoms.

The Dutch translation of the Hospital Anxiety and Depression Scale (HADS)[21] was used to assess psychological distress. The questionnaire contains 14 items on emotional state during the past week which have to be answered on a four-point scale. Because somatic symptoms of anxiety and depression do overlap with somatic symptoms of a disease (or with FS symptoms), regular mood questionnaires are often not valid in a somatic population. The HADS is specifically designed for assessment of anxiety and depression levels in persons with somatic symptoms. Reliability of the Dutch translation of the questionnaire is acceptable with cronbach's coefficient alpha 0.81-0.84 for the anxiety subscale and 0.71-0.86 for the depression subscale[21].

Cluster analyses

Respondents were clustered on their scores on the 47 somatic symptoms with a *k*-means cluster analysis, using SPSS for Windows, version 20.0.0. The *k*-means cluster analysis is an iterative partitioning method in which the number of desired clusters is set a-priori. After an initial randomly generated partitioning of the cases, they are assigned in an iterative manner to the group with the nearest centroid. Cluster centroids are recalculated after every step and cluster assignment is repeated until convergence is achieved[22]. Screening of the individual scores on the somatic complaint variables yielded two outliers. Because of the small number of outliers in proportion to the total number of data points, it was decided to retain these in the dataset. Three cluster analyses were performed with a-priori settings of 2, 3 and 4 clusters. In contrast with other studies using cluster analyses, no a-priori symptom groups were created. Instead, all symptoms were entered in the analyses separately.

Cluster comparisons

Clusters in all three cluster solutions were compared on total symptom scores and cluster-specific symptom patterns. Clusters were also compared on anxiety and depression scores on the HADS and on demographic variables. Differences between clusters in total symptom scores, HADS scores, age, and BMI were tested with independent t-tests for the 2-cluster solution and analyses of variance (ANOVA) for the 3- and 4-cluster solutions. ANOVA's were followed by post-hoc t-tests with Bonferroni correction for multiple comparisons. Differences in gender ratio were analyzed with chi-squared tests (χ^2), with post hoc interpretation of the standardized residuals to assess which cell contributed to the statistic (a cell with a standardized residual above 1.95 can

be interpreted as contributing to the statistic). Finally, max C(K) (a variance ratio criterion) was calculated for each of the cluster solutions as an indicator for the best number of clusters with the following formula: (trace B/(K-1))/trace W/(n-K). A higher value of C indicates a better ratio of within and between cluster variance and thus an indication for the optimal number of clusters[23].

Results

FSS characteristics of the sample

Mean score on the symptom list was 2.31 (SD: 0.62), with a range of 1.06 - 4.53. None of the respondents reported *not* to have experienced any of the symptoms in the last week. Mean scores on the separate items of the symptom list ranged between 1.29 ('fainting') and 3.93 ('feeling tired'). All items had a range of 1 - 5.

Diagnoses of functional somatic syndromes were reported for fibromyalgia (FM) (17.5%), chronic fatigue syndrome (CSF) (9.9%), irritable bowel syndrome (IBS) (3.8%), atypical facial pain (0.5%), hyperventilation (0.3%), and whiplash (0.3%). A small number of respondents reported a diagnosis of more than one FS syndrome: 1.3% CFS and FM, 1.8% FM and IBS. Over 64 percent of the total sample did not report having a diagnosis of an FS syndrome.

Cluster solutions and total symptom scores per cluster

Cluster solutions for k = 2, 3, and 4 are shown in Table 1, with the columns displaying the average scores of the subjects in this cluster on the 47 symptoms and on all symptoms combined. In all three solutions the clusters show increasing total symptom scores from cluster A up to cluster B, C or D (p's <.001), except for the increase in total symptom score between clusters 4-B and 4-C, which was not significant (p = .35).

Cluster-specific symptom patterns

Cluster-specific symptom patterns were searched for by examining mean scores per symptom group per cluster (Figure 1). In the 2-cluster solution, clusters 2-A and B differed in general level of symptoms. Between cluster comparisons showed a significant difference in severity score for all symptom groups between the two clusters (F (7,386) = 100.49, p < .001; p-values for post-hoc tests all < .001). The clusters showed a remarkably similar pattern of symptoms: a relatively high score on fatigue, cognitive, and

musculoskeletal complaints as compared to the other symptom groups (Figure 1, Graph 1).

The 3-cluster solution showed the same type of results: an overall difference in severity of symptoms (Figure 1, Graph 2). Visual inspection revealed that clusters 3-B and C were specifically characterized by relatively high scores on fatigue and cognitive complaints as compared to the other symptom groups. Between cluster comparisons showed a significant difference in severity score between clusters 3-A and B for all groups of symptoms except gastrointestinal symptoms (F (14,770) = 96.24, $p < .001$; post hoc comparisons $p < .001$ for fatigue, cognitive, cardiac, respiratory, musculoskeletal, and 'other' symptoms, post hoc comparison for gastrointestinal symptoms $p = .06$), while cluster 3-C had higher symptom scores on all symptom groups, compared to clusters 3-A and B (all post hoc comparisons $p < .001$).

In the 4-cluster solution, specifically cluster 4-B showed a relative predominance of musculoskeletal complaints (Figure 1, Graph 3). In the three clusters with higher overall symptom scores (4-B, C, and D), scores on fatigue and cognitive complaints were higher relative to the other symptoms groups. Between cluster comparisons showed lower symptom scores in cluster 4-A as compared to B for all symptom groups except for respiratory complaints (F (21,1103.19) = 83.30, $p < .001$; post hoc comparisons $p < .001$ for fatigue, cognitive, cardiac, musculoskeletal, gastrointestinal, and 'other' complaints groups, post hoc comparison for respiratory complaints $p = .08$). Clusters 4-B and C differed in fatigue, cognitive, and musculoskeletal complaints only (p's < .001), with higher scores on musculoskeletal complaints in cluster 4-B and higher scores on fatigue and cognitive complaints in 4-C. Clusters 4-C and D differed on all groups of symptoms (p's < .001).

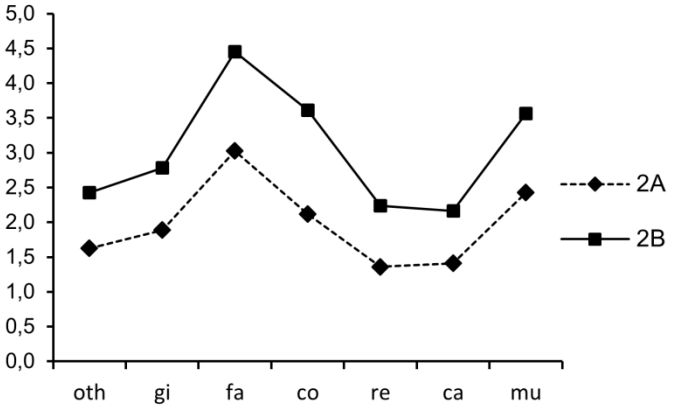
Thus, all three cluster solutions showed that with increasing symptom severity, symptom scores increase in most symptoms groups, with a greater increase in fatigue and cognitive complaints. Further, when the cluster solution allows for intermediate mean symptom scores, such as in the 4-cluster solution, cluster differences in symptom patterns show up. For the cluster with a high level of complaints (cluster 4-D) the pattern of complaints is not so much different from those with low level of complaints (4-A) but at intermediate levels of complaints specifically one cluster shows up (4-B) with musculoskeletal symptoms.

Table 1. Total and individual symptom scores per cluster within cluster solution.

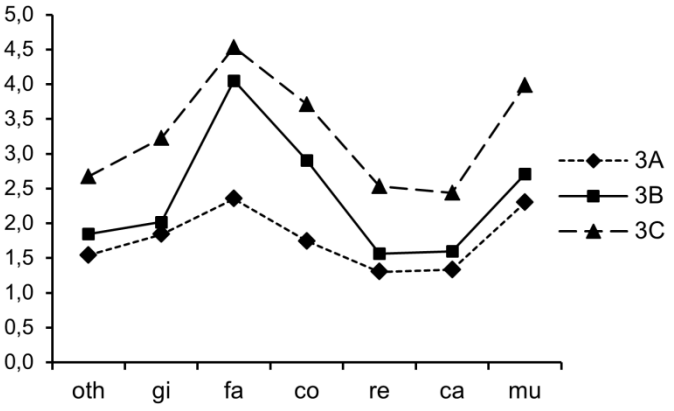
		2-cluster solution		3-cluster solution			4-cluster solution			
	Cluster names	2-A	2-B	3-A	3-B	3-C	4-A	4-B	4-C	4-D
	n	251	143	142	167	85	115	98	110	71
	Total symptom scores (SE)	1.94 (.02)	2.96 (.04)	1.74 (.03)	2.33 (.02)	3.21 (.04)	1.66 (.03)	2.31 (.03)	2.35 (.03)	3.29 (.05)
Symptom groups	Symptoms									
Gastrointestinal	Upset stomach	1.70	2.64	1.63	1.84	3.13	1.56	2.04	1.85	3.14
	Abdominal pain or stomach pain	1.90	2.72	1.92	1.92	3.24	1.82	2.23	1.87	3.28
	Bowel cramps	1.91	2.69	1.85	2.04	3.07	1.81	2.16	2.05	3.10
	Bloated stomach	2.04	3.08	1.96	2.27	3.47	1.89	2.43	2.30	3.45
Fatigue	Feeling low on energy	3.37	4.69	2.67	4.41	4.72	2.61	3.85	4.55	4.77
	Feeling tired	3.45	4.76	2.72	4.51	4.79	2.67	4.01	4.58	4.83
	Feeling exhausted	3.11	4.65	2.24	4.37	4.68	2.17	3.70	4.51	4.76
	Feeling physically weak	2.81	4.41	2.06	3.96	4.49	1.99	3.36	4.12	4.58
	Not feeling fit	3.39	4.66	2.71	4.38	4.71	2.55	3.98	4.51	4.76
	Feelings of muscle weakness	2.01	3.53	1.70	2.67	3.80	1.56	2.94	2.42	3.90
Cardiac	Chest pain	1.41	2.25	1.32	1.60	2.61	1.26	1.70	1.57	2.69
	Rapid heart beat	1.53	2.36	1.43	1.79	2.59	1.43	1.61	1.90	2.69
	Pounding heart	1.44	2.28	1.39	1.65	2.53	1.36	1.65	1.70	2.58
	Tightness around the chest	1.40	2.13	1.35	1.57	2.38	1.30	1.54	1.63	2.48
	Irregular heartbeat	1.37	2.11	1.31	1.54	2.39	1.27	1.63	1.51	2.46
	Painful stings in the heart area	1.29	1.85	1.20	1.42	2.14	1.15	1.42	1.46	2.21
Respiratory	Feelings of dyspnea	1.39	2.36	1.34	1.62	2.67	1.37	1.48	1.65	2.87
	Shortness of breath	1.57	2.48	1.42	1.86	2.79	1.39	1.73	1.92	2.93
	Inability to take a deep breath	1.35	2.38	1.31	1.59	2.69	1.34	1.44	1.70	2.80
	Sudden fast or deep breathing	1.23	1.89	1.21	1.33	2.16	1.20	1.23	1.41	2.31
	Breathlessness	1.25	2.07	1.23	1.41	2.35	1.26	1.26	1.49	2.51
Musculoskeletal	Muscle pain	2.87	4.02	2.73	3.20	4.39	2.33	4.32	2.64	4.42
	Pain in bones	2.01	3.06	1.94	2.20	3.51	1.62	3.24	1.68	3.55

	Pain in joints	2.43	3.81	2.29	2.84	4.20	1.87	4.04	2.22	4.24
	Back pain	2.43	3.40	2.31	2.64	3.85	2.04	3.62	2.15	3.79
	Pain in neck	2.53	3.47	2.40	2.75	3.89	2.13	3.54	2.38	3.90
	Stiffness of fingers, arms, or legs	2.28	3.62	2.13	2.63	4.11	1.76	3.67	2.12	4.17
Cognitive	Difficulty concentrating	2.41	4.03	1.92	3.37	4.06	1.87	2.47	3.87	4.20
	Forgetfulness	2.12	3.58	1.73	2.84	3.82	1.77	2.03	3.26	3.97
	Having trouble paying attention	2.23	3.85	1.80	3.13	3.91	1.78	2.19	3.67	4.03
	Unclear or foggy thoughts	1.95	3.66	1.51	2.89	3.73	1.50	1.90	3.44	3.89
	Distracting thoughts	2.23	3.63	1.95	2.95	3.64	1.95	2.36	3.22	3.80
	Confusion or feelings of unreality	1.77	2.92	1.55	2.26	3.13	1.55	1.80	2.54	3.24
Other	Excessive sweating	1.62	2.79	1.49	2.02	3.01	1.47	1.79	2.13	3.20
	Hot or cold flashes	1.91	3.36	1.77	2.51	3.40	1.75	2.30	2.57	3.54
	Dry mouth	1.71	2.66	1.59	2.04	2.86	1.52	1.97	2.14	2.92
	Headache	2.30	2.98	2.06	2.61	3.22	2.01	2.61	2.61	3.23
	Trembling of hands, arms, or legs	1.40	2.33	1.33	1.64	2.61	1.33	1.61	1.69	2.65
	Tingling feeling in fingers, arms, or legs	1.80	2.69	1.73	1.98	3.05	1.66	2.19	1.87	3.14
	Numb feeling somewhere in body	1.55	2.58	1.50	1.74	2.98	1.37	1.97	1.74	3.03
	Nausea	1.61	2.31	1.55	1.73	2.67	1.52	1.82	1.76	2.66
	Fainting	1.16	1.54	1.09	1.26	1.71	1.09	1.21	1.29	1.75
	Having trouble swallowing	1.24	1.80	1.16	1.39	2.01	1.16	1.41	1.39	2.03
	Sore throat	1.39	1.94	1.29	1.53	2.19	1.25	1.58	1.58	2.14
	Rustling sound in ears	1.85	2.26	1.88	1.84	2.49	1.89	1.94	1.79	2.58
	Lump in throat	1.47	2.18	1.41	1.58	2.54	1.28	1.79	1.61	2.55
	Dizziness	1.76	2.53	1.70	1.98	2.71	1.71	1.71	2.20	2.76

Graph 1



Graph 2



Graph 3

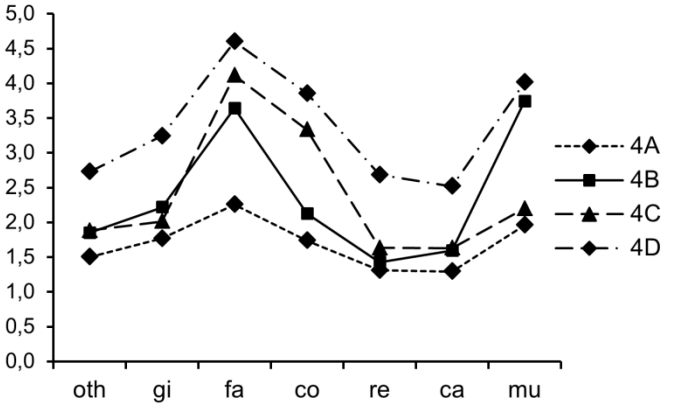


Figure 1. Cluster means per symptom group for the 2-cluster solution (Graph 1), 3-cluster solution (Graph 2), and 4-cluster solution (Graph 3). gi = gastrointestinal; fa = fatigue; co = cognitive; re = respiratory; ca = cardiac; mu = musculoskeletal; oth = other complaints.

Cluster differences on demographics

See Table 2 for descriptive information on age, BMI, and gender for all three cluster solutions. Age differed between clusters. In all cluster solutions, persons in the clusters with relatively low symptom scores were older compared to the other clusters (*p*'s < .012). BMI did not differ between clusters in either of the solutions (*p*'s > .05). Proportion of females did not differ within the 2- and 3-cluster solution (both *p*-values > .05), and although an overall association was found between cluster-membership and gender within the 4-cluster solution ($\chi^2(3) = 8.23$, $p = .04$), none of the cells made a significant contribution to the chi-square statistic.

Table 2. Descriptives per cluster solution.

	2-cluster solution		3-cluster solution			4-cluster solution			
	2-A	2-B	3-A	3-B	3-C	4-A	4-B	4-C	4-D
Age	50.55*	44.70	51.74*	47.59	44.55	51.53[a]	49.94	46.06	45.00
(SE)	(.77)	(1.11)	(.97)	(.98)	(1.53)	(1.11)	(1.27)	(1.15)	(1.74)
BMI	24.68	25.08	24.40	25.00	25.21	24.07	25.33	24.88	25.29
(SE)	(.29)	(.49)	(.31)	(.47)	(.56)	(.32)	(.48)	(.62)	(.62)
% females	76.5	75.5	76.1	73.7	81.2	73	84.7	69.1	80.3

* Different from other clusters within solution with $p < .017$.
[a] Different from clusters 4-C and 4-D with $p < .008$.

Cluster differences on anxiety and depression

The HADS anxiety and depression subscale scores differed between clusters in all three cluster solutions (Figure 2) (2-cluster: $t(252.35) = -7.88$, $p < .001$ and $t(392) = -8.85$, $p < .001$, 3-cluster: $F(2,391) = 31.55$, $p < .001$ and $F(2,391) = 52.22$, $p < .001$, and 4-cluster: $F(3,390) = 21.82$, $p < .001$ and $F(3,390) = 44.79$, $p < .001$). Within all cluster solutions, anxiety and depression scores increased linearly over the clusters (*p*'s < .001), with some exceptions within the 4-cluster solution. Here, anxiety did not differ between clusters 4-A and B ($p = .13$) and between clusters 4-B and C ($p = .51$). Depression scores did not differ between clusters 4-C and D ($p = .23$).

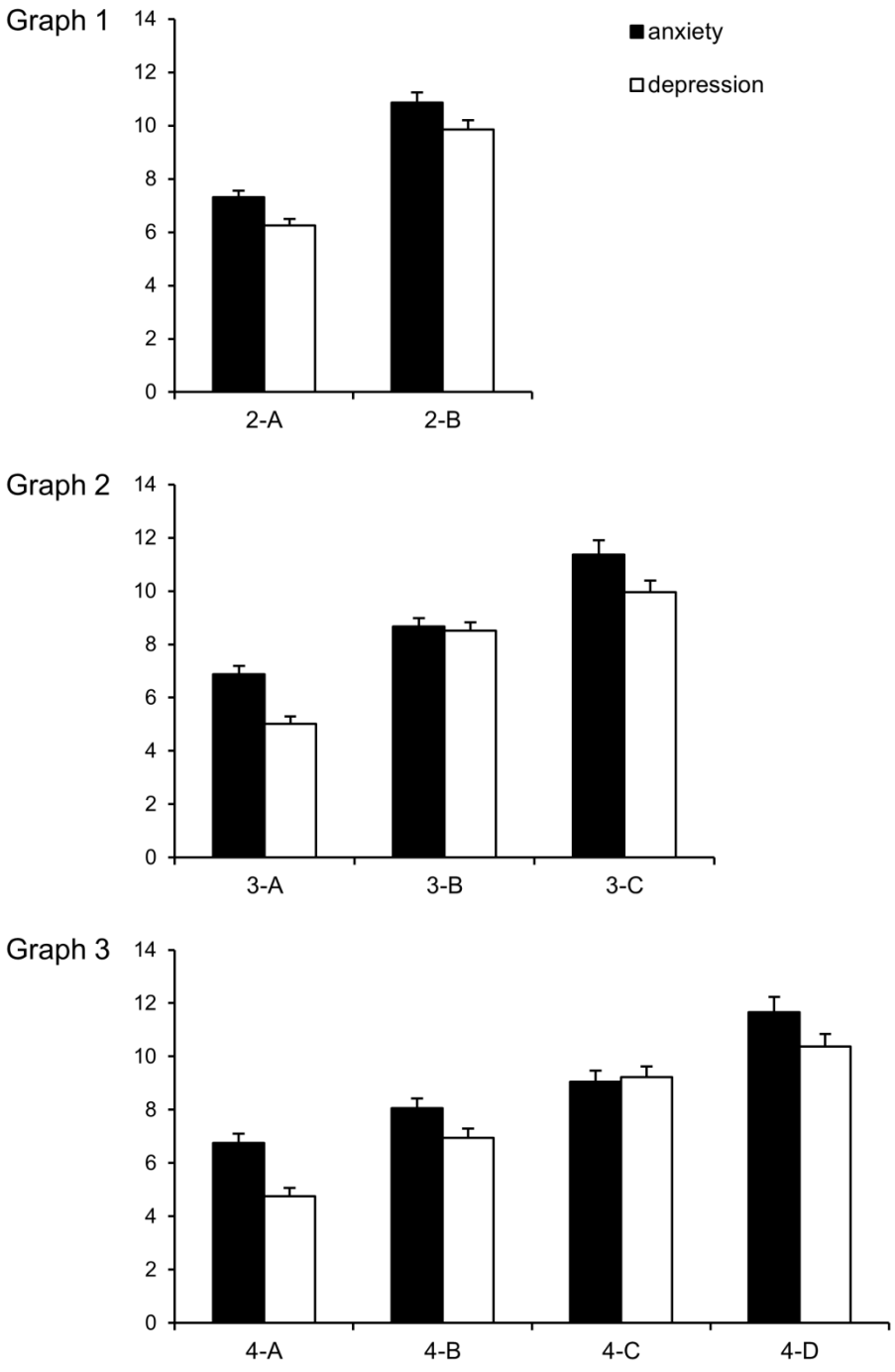


Figure 2. Mean HADS anxiety and depression score per cluster for the 2-cluster solution (Graph 1), the 3-cluster solution (Graph 2), and the 4-cluster solution (Graph 3). *: different from all other clusters within solution with $p < .001$. #: different from cluster 4-A with $p < .001$.

Comparison of solutions on fit index

The variance ratio criterion C(K) was 36110 (4969/0.137) for the 2-cluster solution, 26620 (3372/0.126) for the 3-cluster solution, and 22201 (2651/0.119) for the 4-cluster solution. Changes in C across the solutions could be explained mainly by changes in the numerator: between cluster variance increased over the solutions, but the numerator decreased after controlling for number of clusters. Thus, clusters could best be discriminated in the 2-cluster solution.

Discussion

The current study yielded three major findings. First, in all *k*-means cluster solutions, clusters were defined by increasing total symptom scores and increasing psychological distress. Second, cluster-specific symptom patterns were found only when more (three or four) clusters were allowed. Third, clusters could best be discriminated in the 2-cluster solution.

Our finding that total symptom severity score distinguished adequately between clusters in all three cluster solutions is similar to the findings of previous cluster studies where clusters were most adequately differentiated by the number of symptoms[14,17,18]. Remarkably, the two clusters with specific symptom patterns in the 4-cluster solution (i.e. 4-B and C) did not differ in total symptom score, indicating that within a range of intermediate symptom scores, specific differentiation on symptom patterns occurs. Anxiety and depression scores were positively related to the total symptom score (as has been found before[24,25] in all cluster solutions. However, in the 4-cluster solution this relation becomes less clear, with no difference in depression between cluster 4-C and D and anxiety mostly differing between cluster 4-D and the other clusters. Possibly, the two clusters with intermediate symptom scores diffuse the overall relation between psychological distress and symptom scores. Overall, the clear association between depression/anxiety and symptom score favors the lumpers' position.

In all three cluster solutions, persons in the clusters with low overall symptom scores were older compared to the other clusters. This finding is in line with research on the relation between age and symptom experience in fibromyalgia where lower symptom severity was found in older age groups[26,27], and in line with population surveys showing a lower prevalence of fatigue in older subjects (here the most prevalent complaint)[28]. A

possible explanation could be that older persons have better coping strategies, resulting in the symptoms to be perceived (and reported) as less intense.

Our findings on multiple cluster solutions can be used to interpret the different outcomes of previous cluster- or class analyses. Our 4-cluster solution resembles the solutions reported by Gara et al. and Kato et al.[17,18], where respectively eleven and five clusters or classes were found, which could be defined either by specific symptom groups or by increasing total symptom counts. Our 2-cluster solution resembles the solution by Fink et al.[14], who found either two or three classes, which differed only in the number of symptoms. Thus, our results show that an a-priori selection of fewer clusters in a solution results in finding only the (probably stronger) differences in overall symptom severity or symptom count. When a solution with more clusters is selected, more subtle differences between patients in symptom patterns become visible. However, according to our fit index, a solution with fewer (i.e. two) clusters is preferred above solutions with more clusters (i.e. three or four), thus favoring the lumpers' side of the debate.

Although more support was found in the current study for the lumpers' perspective, results of our three cluster solutions provide evidence for both sides of the lumpers-splitters argument and thereby support earlier findings of latent class analyses. As has been suggested in a recent review by White[12], possibly both sides are true in that there is mainly commonality but to a certain extent also heterogeneity between FS syndromes. Results from treatment studies of FS syndromes also give support for both sides of the argument, as some treatments (e.g. psychotherapy) seem to have effect across FS syndromes, while others (e.g. pharmacotherapy) are effective in only some[29]. Thus, we do not recommend dismissing the evidence for the splitters' perspective. FS symptoms should be regarded from both perspectives in research and treatment settings.

Limitations

An internet cohort was used in this study recruited through FS patient sites. This procedure may have resulted in a non-representative sample consisting of persons who are: 1) active on the internet, 2) willing to engage in an online survey, and 3) probably actively seeking information about their FS symptoms on the internet. Because the respondents have not been clinically screened for possible medical explanations for their symptoms, we cannot be absolutely sure all symptoms they reported were

medically unexplained. This patient group, however, is known for frequent visits to medical doctors. Earlier studies have recruited participants through medical care facilities, thereby including only patients that were (still) actively seeking medical care for their symptoms, introducing another type of selection-bias. Our internet recruitment-approach adds to these results by including participants who are not necessarily (still) active in the medical system. Based on the self-reports of syndrome diagnoses, we conclude that we mostly reached persons with (complaints resembling) chronic fatigue syndrome and fibromyalgia. This may have limited our findings as well.

Conclusion

In the current study, we found support for both the lumpers' and the splitters' perspective. In favor of the lumpers' perspective, the clusters within our solutions differ from each other in total symptom score, and in each cluster solution a group with a large range of symptoms and a group with low score on all symptoms was found. For these groups, no symptom patterns could be discerned and splitting seems to be of no use. Further, the variance ratio criterion indicated that patient groups could best be discriminated from each other when only divided on overall symptom severity. In favor of the splitters' perspective, we found that by forcing a four-cluster solution, persons with intermediate symptom scores could be further classified based on specific symptom patterns. Weighing the support for the splitters and for the lumpers, we conclude that the results of our study are more in favor for the lumpers' perspective of the argument, although both sides are probably true.

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Chapter 3

Experimental pressure-pain assessments: Test-retest reliability, convergence and dimensionality

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Scandinavian Journal of Pain, 2012; 3, 31-37

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Abstract

Objective: Experimental pain studies can provide unique insight into the dimensions of pain and into individual differences in pain responsiveness by controlling different aspects of pain-eliciting stimuli and pain measures. In experimental pain studies, pain responsiveness can be assessed as pain threshold, pain tolerance or pain ratings. The test-theoretical qualities of these different measures, however, have not yet been completely documented. In the current study, several of these qualities were investigated in a pain experiment applying different algometric techniques. The objective of the study was to investigate the reliability (test–retest) and the convergent validity (correspondence) of the different methods found in the literature of measuring pressure-pain threshold, and the interrelationship between pressure-pain threshold, pressure-pain tolerance, and pressure-pain ratings. ***Methods:*** Sixty-six healthy female subjects were enrolled in the study. All pressure stimuli were applied by a trained investigator, using a digital algometer with a 1 cm^2 rubber tip. Pressure-pain thresholds were assessed repeatedly on six different body points (i.e. left and right calf one third of total calf muscle length below the popliteal space), the lower back (5 cm left and right from the L3), and left and right forearm (thickest part of brachioradialis muscle). Next, pressure-pain tolerance was measured on the thumbnail of the non-dominant hand, followed by rating affective and sensory components (on visual analogue scales) of a stimulus at tolerance level. Last, affective and sensory ratings were obtained for two pressure intensities. ***Results:*** With intraclass correlations above .75 for pain responses per body point, test–retest reliability was found to be good. However, values obtained from all first measurements were significantly higher as compared to the two succeeding ones. Convergent validity of pain thresholds across different body points was found to be high for all combinations assessed (Cronbach's alpha values > .80), but the highest for bilateral similar body parts (> .89). Finally, principal components analysis including measures of threshold, tolerance and pain ratings yielded a three-factor solution that explained 81.9% of the variance: *Moderate-level stimulus appraisal & pain tolerance; Pain threshold; Tolerance-level stimulus appraisal*. ***Conclusion:*** Findings of the current study were used to formulate recommendations for future algometric pain studies. Concerning pressure-pain threshold, it is recommended to exclude first measurements for *every* body point from further analyses, as these measurements were found to be consistently higher compared to the following measurements. Further, no more than two consecutive measurements (after the first

measurement) are needed for a reliable mean threshold value per body point. When combining threshold values of several body points into one mean-aggregated threshold value, we suggest to combine bilateral similar points, as convergent validity values were highest for these combinations. The three-factor solution that was found with principal components analyses indicates that pressure-pain threshold, subjective ratings of moderate intensity stimuli, and subjective ratings of the maximum (tolerance) intensity are distinct aspects of pain responsiveness. It is therefore recommended to include a measure of each of these three dimensions of pain when assessing pressure pain responsiveness. Some limitations of our study are discussed.

Introduction

Experimental pain studies can provide unique insight into the dimensions of pain and into individual differences in pain responsiveness by controlling different aspects of pain-eliciting stimuli and pain measures. In experimental pain studies measures based on the stimulus intensity are frequently used, such as pain threshold (i.e. the stimulus intensity at which a person first experiences pain) and pain tolerance (i.e. the stimulus intensity at which a person perceives the pain as unbearable). Pain responsiveness can also be assessed by pain ratings, for example pain intensity rating on a visual analogue scale. This diversity in experimental measures of pain responsiveness raises questions about the validity and reliability of these measures and their interrelationship. More insight in these issues is needed to raise experimental pain studies to a higher level.

In addition to a diversity in stimulus intensity and pain measures, different modalities of pain stimuli have also been used across studies, such as heat and cold stimuli, electrocutaneous stimuli and pressure stimuli. These stimuli do not elicit the same pain responses[1] and outcome measures between these modalities are not always related[2]. Among these modalities, pressure (i.e. algometry) has most frequently been used for comparing pain perception between pain patients and (healthy) controls[3-6]. The use of algometry in the diagnosis of rheumatic diseases indicates that pressure pain is considered a relevant experimental model for clinical pain experienced by these patients[7,8,9]. Other studies have demonstrated that pressure-pain perception measures are also related to clinical pain in fibromyalgia patients and in women with a wide range of pain complaints, while other stimulus modalities are not[10-12]. Because pressure pain is the only modality of which the clinical relevance has been demonstrated, the focus of the current validity and reliability study will be on algometric measures.

Experimental pressure-pain studies aimed at demonstrating differences between pain patient groups and controls often show inconsistent results[13,14,15], which can possibly be attributed to the chosen methodology of pain assessment. Although the influence of some variations in methodology on study outcomes have already been addressed[4,12,16], there remain some fundamental issues that will be addressed in the current study. First, the pressure-pain threshold on a single body point is often assessed with repeated measures. Pain threshold is then either defined as the mean-aggregated values of (some of) these measurements[3,11], or the pressure on the last measurement[17]. Specific decisions in this procedure of data analysis, however, may

have large consequences for the results. It is not clear whether using repeated measures of pain threshold (or pain tolerance for that matter) on a single point yield reliable values, and if so, how many repetitions should be performed to get a robust measure of pain threshold. Although the measurement of pressure-pain tolerance could raise the same questions, this measure is not often measured repeatedly probably because of ethical considerations. Therefore, we will restrict this issue to threshold measures only.

The second issue deals with the different methodologies found in the literature in which pressure-pain thresholds are measured on multiple body parts, often bilaterally. For example, threshold is measured on both the left and the right arm and also on the left and right calf. Data are either collapsed into a mean-aggregated threshold over the bilaterally assessed body part (e.g. the left and right arms)[2] or into a mean-aggregated overall threshold including all body parts (e.g. both arms and legs)[4]. Although it has been shown that there is no statistical difference in pain threshold between dominant and non-dominant site per body part[3], it is unknown what the correspondence is between pressure-pain thresholds on different body parts and whether this procedure masks area-specific effects.

The third and final issue addressed in the current study focuses on the difference between using pressure-pain thresholds and tolerance levels versus using pain ratings as the main outcome. In a typical experimental pressure-pain threshold or tolerance study, participants undergo several stimulus intensities. Next, the intensity of stimulus threshold or tolerance (in for example kilopascal) is used as outcome and compared between individuals. In a typical pain-rating study, self-reported intensity or unpleasantness of pain (using a VAS or Likert scale) of a predefined stimulus intensity is compared between individuals. However, tests for differences in pressure-pain threshold and tolerance may yield different results than tests for differences in ratings of stimuli. It has already been demonstrated that pressure-pain thresholds based on *ratings of stimulus intensity* yielded different results than pressure-pain thresholds based on *the first indication that the stimulus became painful*[4]. There is also evidence that intensity and unpleasantness ratings do relate to clinical pain measures differently than pain threshold and tolerance levels[10]. These measures may very well reflect distinct aspects of pain.

The objective of the current study was to investigate the reliability (test–retest) and the convergent validity (correspondence) of the different methods found in the literature

of measuring pressure-pain threshold, and the interrelationship between pressure-pain threshold, pain tolerance, and pain ratings. Specifically, the following questions will be answered: a) what is the reliability of repeated measurements of pressure-pain threshold on a single body point, b) what is the correspondence between pressure-pain thresholds across different body parts, and c) to what extent are pressure-pain threshold, pressure-pain tolerance, and pain ratings aspects of a common dimension or of separable dimensions. Based on the results of the current study, methodological recommendations for future experimental algometric pain studies will be formulated.

Methods

Subjects

Eligible female undergraduates were selected based on their answers on an online survey on health and health behavior. Only females were included in the sample to increase homogeneity. The online survey was advertised on posters and flyers which were distributed throughout the university campus. A total of 370 female students filled in the online survey, of which 263 gave permission to be invited for the current study. One hundred-eleven respondents were excluded based on having a medical condition (including having the flu or a cold), using prescribed medication (including frequent use of asthma medication, not including contraceptives), or both. From the 152 respondents who were invited to participate, 66 showed up. All subjects participated in exchange for remuneration or course credit.

Instruments

All pressure stimuli were applied by a trained investigator. A digital algometer (FDX 50; Wagner Instruments) was used with a 1 cm^2 rubber tip that was placed on the skin or finger nail. Pressure was recorded in kilopascal (kPa), with the algometer recording graduations of 1.96 kPa.

For pressure-pain tolerance, an additional holding device was designed with which the pressure on the thumb nail could be applied in a more controlled manner. The algometer was placed in a wooden casket, which could be moved up and down inside a wooden column. Participants inserted their thumb into the column underneath the algometer, after which the algometer could be pressed down manually by the investigator by moving a lever down. Safety blocks inside the column made sure the

casket could not be moved down entirely, thus ensuring enough space for the thumb. This device was also used in applying discrete stimuli for assessment of pressure-pain ratings.

Pressure-pain measures

An overview of the pressure-pain measures assessed in the current study is given in Table 1.

Pressure-pain threshold

In the current study, pressure-pain threshold was defined as the pressure at which the participant first indicated the pressure to become unpleasant. The expression "unpleasant" was chosen to avoid possible anxiety effects of the expression "painful". During pressure-pain threshold (PPth) measurement, the participant was in a prone position on a massage table with the head facing down in a face rest. Pressure was applied using the algometer and gradually increased with 98 kPa per second until the participant indicated that the pressure became unpleasant by saying "stop", after which the algometer was immediately removed from the skin and maximum pressure was copied from the algometer screen. To enable the investigator to increase the pressure at a constant rate, a digital metronome was used to indicate one-second time interval (with soft ticking sounds). The use of the metronome was explained to the participants as a tool to help the investigator with the measurements, but it was not explained how this was used.

PPth was measured three times on six body points in a fixed order without breaks: left and right calf (one third of total calf muscle length below the popliteal space), the lower back (5 cm left and right from the L3), and left and right forearm (thickest part of brachioradialis muscle) respectively. On each body point, the three measurements were taken successively before moving to the next point. Time intervals between measurements were 30 - 40 seconds. Pressure did not exceed 1471 kPa at the legs or the lower back or 980.5 kPa at the arms. When PPth was not indicated before reaching these pressures, 1471 or 980.5 kPa was noted down as the maximum pressure.

Pressure-pain tolerance

Pressure-pain tolerance was defined as the maximum pressure at which the participant indicated the pressure to become too painful. Pressure-pain tolerance (PPtol) was assessed on the thumb of the non-dominant hand with the subject sitting in

a chair using the holding device described above. Pressure was increased with 98 kPa per second until the subject indicated the pressure to become too painful. After each trial, the subject was asked whether or not that point was actually reached. If not, the subject was asked to try again. Inter-stimulus times were 30 - 40 seconds. Maximum pressure was recorded for every trial. Pressure did not exceed 1471 kPa. In case PPtol was not indicated at 1471 kPa, this pressure was noted down as PPtol.

Pressure-pain ratings

For several stimulus intensities, two pressure-pain sensations were rated. Unpleasantness (affective aspect) of the pressure stimulus was rated on a 100 mm visual analogue scale ranging from “not unpleasant at all” to “the most unpleasant feeling ever”, while painfulness (sensory aspect) was rated on a 100 mm scale ranging from “not painful at all” to “the most painful feeling ever”. First, the participant was asked to rate the sensation of the pressure pain tolerance stimulus on the thumb of the non-dominant hand for the last trial of the tolerance measurement (i.e. the trial on which the participant indicated that the pressure-pain tolerance level was reached). Second, discrete stimuli of 294 kPa and 490 kPa were applied to the thumb of the dominant hand and the participant was instructed to rate the sensation of these stimuli directly after the pressure stimulus was applied. Pressure was again increased with 98 kPa per second until the desired level was reached. On reaching either 294 or 490 kPa, pressure was maintained for two seconds after which the algometer was removed from the nail.

Table 1. Overview of pressure-pain measures and methods.

Pressure-pain measures	Procedure
Pressure-Pain Threshold (PPth)	Three measurements on left and right calf, lower back, and forearm
Pressure-Pain Tolerance (PPtol)	Maximal tolerance of thumb pressure
Pressure-Pain Ratings	Unpleasantness and painfulness of thumb pressure maximal (tolerance), 294, and 490 kPa

Procedure

Pressure-pain measures were taken between 9 am and 5 pm in a laboratory setting. Subjects were scheduled according to their own preference and were asked to refrain from caffeine intake in the 2 hours before participating. See Figure 1 for a time line of

the procedure. After obtaining informed consent, pressure pain perception was assessed in the following order: PPth, PPtol, subjective ratings of a stimulus at tolerance intensity and of discrete pressure stimuli, with short breaks (i.e. 1 - 3 minutes) between measures. Verbal instructions were given before every measurement, followed by practice trials. The protocol was in accordance with the principles of the Declaration of Helsinki (October, 2008).

At the end of the session, subjects were asked to fill out a questionnaire booklet, containing questions regarding current pain complaints, current medical condition (which started after the internet survey) and current medication use, as well as control questions regarding caffeine intake in the 2 hours preceding the study, sleep during the last two nights and amount of intensive physical exercise on the day preceding the study or on the day of the study itself.

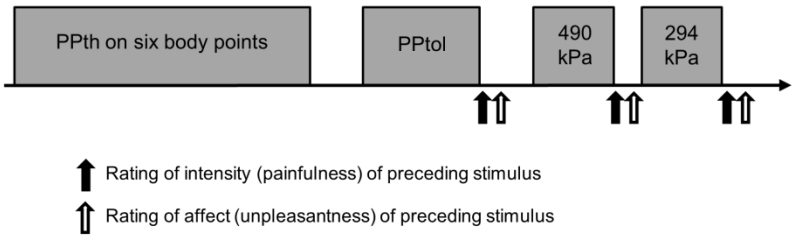

Figure 1. Time line of assessment of pressure-pain measures.

Statistical analysis

All measures were tested for normality and presence of outliers and, when needed, appropriate transformations (i.e. logarithmic) were applied. For analyzing test–retest reliability of the repeated measures of PPth per body point, intraclass correlation coefficients (ICC) in a two way mixed model with type ‘absolute agreement’ were calculated. The correspondence between PPth measures across different body parts was assessed with reliability analysis using Cronbach’s alpha. To investigate to what extent pain tolerance, pain thresholds, and pain ratings entail different aspects of pain responsiveness, the factor structure of the pressure-pain measures (i.e. PPth, PPtol and subjective ratings of tolerance intensity and of 294 and 490 kPa stimuli) was investigated with principal components analysis. The number of factors was determined by examining the eigenvalues of the factors, where factors with eigenvalues ≥ 1 were

retained. Since factors were expected to correlate with each other, oblique (oblimin) rotation was performed before interpreting the factor solution. All analyses were performed with SPSS 16.0 for Windows[18].

Results

Subjects

A sample of 66 female undergraduates participated in the study, with a mean age of 21.53 (SD: 3.47) and mean body mass index of 21.99 (SD: 2.52). Twelve subjects (18.2% of the sample) indicated to have a cold or to have mild flu-like symptoms on the day of study participation, although none of them felt too unwell to participate. Twenty-one subjects (31.8 %) reported pain complaints in one or several body parts, of which 11 indicated pain in neck, shoulders, or upper back, and 4 reported pain in the middle or lower back. Finally, some participants (n = 9; 13.6%) used medication on the day before participating or on the day of participation: over-the-counter pain inhibitors (n = 4); anti-histamine (n = 2); antibiotics (n = 1); cholesterol synthesis inhibitors (n = 1); and thyroid gland hormones (n = 1). No differences between these subgroups and the remaining sample were found on the pain measures, thus none of the subgroups were excluded.

Pressure-pain measures

Mean pressures for the threshold and tolerance measurements are shown in Table 2. Skewness and kurtosis data were indicative of non-normality for most threshold measures, which was confirmed by tests of normality (Kolmogorov–Smirnov was non-significant for only 4 out of 18 measurements). Log transformation resulted in normality for all measures and less outliers. The log-transformed variables of threshold measures were used for all further analyses.

On average, 2 trials were needed before participants indicated their actual PPtol level was reached (M=1.8, range 1 - 5 trials). When more than one trial was recorded, maximum pressure on the last trial was used as PPtol for that individual. Tests of normality did not yield any deviance from normal distributions and the untransformed variables were used for all further analyses.

Mean sensory and affective ratings are given in Table 3. Skewness and kurtosis data showed a normal distribution of the sensory and affective ratings of the 490 kPa

stimulus and the stimulus at tolerance level. This was confirmed by Kolmogorov-Smirnov tests (all *p*-values > .05). Although both affective and sensory ratings of the 294 kPa stimulus showed non normality, transformations did not result in better distributions, therefore the untransformed variables were used for all further analyses.

Table 2. Mean pressure (kPa) for each pressure pain threshold measurement (1st, 2nd and 3rd) per body point and for pressure pain tolerance.

Body point	Measure	N	Mean (SD, SE)	Range
Pressure pain threshold				
Left calf	1st	65	463.54 (156.29, 19.39)	219.67 – 970.86
	2nd	66	414.84 (151.40, 18.64)	176.52 – 819.84
	3rd	66	392.65 (158.16, 19.47)	141.22 – 908.10
Right calf	1st	66	476.34 (179.63, 22.11)	217.71 – 1149.34
	2nd	66	417.26 (151.63, 18.67)	180.44 – 1017.93
	3rd	66	413.24 (169.44, 20.86)	176.52 – 1176.80
Lower back left	1st	66	474.94 (195.93, 24.12)	125.53 – 1100.31
	2nd	66	449.56 (193.82, 23.86)	123.56 – 1166.99
	3rd	66	453.75 (202.84, 24.97)	117.68 – 1212.10
Lower back right	1st	66	471.88 (185.40, 22.82)	143.17 – 906.14
	2nd	66	454.31 (190.86, 23.49)	166.71 – 963.01
	3rd	66	457.23 (193.96, 23.88)	141.22 – 925.75
Left arm	1st	65	313.64 (117.66, 14.59)	98.07 – 660.97
	2nd	66	285.34 (118.88, 14.63)	103.95 – 662.93
	3rd	66	291.43 (133.74, 16.46)	109.84 – 747.27
Right arm	1st	66	307.14 (127.99, 15.75)	121.60 – 862.01
	2nd	66	281.92 (129.06, 15.89)	129.45 – 947.32
	3rd	66	286.53 (137.30, 16.90)	94.14 – 819.84
Pressure pain tolerance				
Thumb nail	last	66	967.77 (259.05, 31.89)	355.00 – 1465.11

Table 3. Mean ratings of painfulness (sensory) and unpleasantness (affective) of pressure stimuli.

	Sensory rating			Affective rating		
Stimulus intensity	N	Mean (SD)	Range	N	Mean (SD)	Range
Tolerance-level	66	63.0 (17.1)	18.0 – 98.0	66	66.4 (20.3)	12.5 – 100
294 kPa	66	22.4 (23.2)	0.0 – 92.0	66	24.6 (89.0)	0.0 – 89.0
490 kPa	64[a]	34.4 (24.3)	0.0 – 100	64	37.9 (27.5)	0.0 – 100

[a] For two participants pain tolerance level was below 490 kPa; these participants were excluded from rating the 490 kPa pressure stimulus.

Test-retest reliability of repeated measures of pressure-pain threshold

Intraclass correlation coefficients (ICC) for repeated measures per body point are shown in Table 4 (first column). These values were all above .75, indicating good test–retest reliability. However, Figure 2 shows that first measurements on every body point yield higher thresholds compared to second or third measurements, which was confirmed in a repeated measures multivariate analysis of variance where a significant main effect for the three measurements was found (F (2,62) = 44.27, p < .0001). Post hoc paired samples t-tests with Bonferroni corrected p-values (p > .017 indicated significance) indeed showed significant differences in pressure-pain thresholds between 1st and 2nd and between 1st and 3rd measurements on most body points, and no difference between 2nd and 3rd measurements on all body points. Thus, ICCs were also computed for second and third measurements only, resulting in higher ICCs for all body points (Table 4, second column). For further analyses, a mean pressure-pain threshold was calculated per body point, using the log transformed values from the 2nd and 3rd measurements only.

Table 4. Intraclass correlation coefficients (ICC) for repeated pressure-pain threshold measures (log transformed) per body point.

	Repeated measurement combinations	
	1st , 2nd and 3rd	2nd and 3rd
Left calf	.794	.848
Right calf	.758	.808
Lower back left	.896	.926
Lower back right	.940	.952
Left arm	.862	.933
Right arm	.904	.934

Internal consistency of pressure-pain threshold measures across body points

Internal consistency of the mean pressure-pain thresholds across body points was assessed with Cronbach's alpha. Alpha values were computed across: a) bilateral body points (e.g. body points on the left and right arm), b) unilateral body points (i.e. all left body points or all right body points), and c) all body points. See Table 5 for all alpha values. These values were high (above .80) for all comparisons, but somewhat lower

for unilateral combinations than for bilateral combinations or when combining all body points. Thus, results indicate that consistency is higher across bilateral same body parts as compared to unilateral combination. For further analyses, mean PPth's over bilateral body points were calculated.

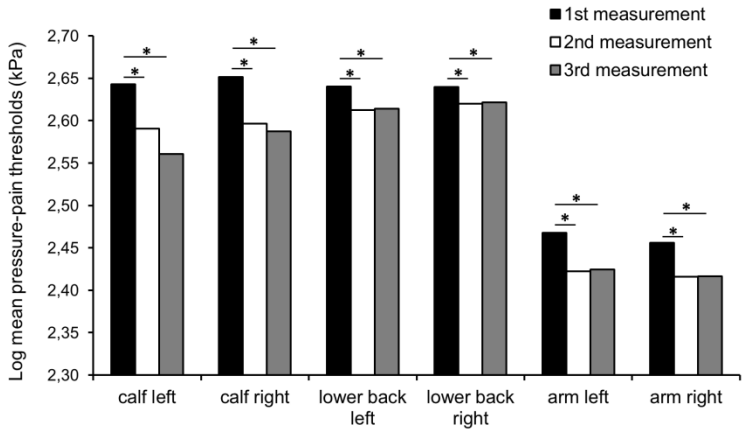

Figure 2. Log mean pressure pain thresholds per measurement and body point. * Significant difference between measurements as analyzed with paired sample *t*-tests ($p < .01$).

Table 5. Cronbach's alpha statistics for mean pressure pain threshold (mean is calculated over log transformed data) measures across combinations of body points (bilateral, unilateral, or all).

		Cronbach's alpha
Bilateral	Leg	.890
	Lower back	.944
	Arm	.948
Unilateral	Left	.814
	Right	.872
All		.931

Interrelationship of pressure-pain measures

To investigate to what extent PPth, PPtol, and subjective ratings of tolerance level, 294, and 490 kPa are distinct aspects of pain responsiveness, principal components analysis (PCA) was performed including all pain measures. First, suitability of the data for PCA was assessed. All pain measures were checked for univariate outliers by inspecting standardized scores (*z* scores). *Z* scores above 3.0 were considered

outliers. No outliers were detected in the variables. Table 6 shows correlations between all pain variables. Several correlations were .30 or above, indicating that inter-correlations were sufficiently high to be suitable for PCA. This was confirmed by Bartlett's test of sphericity, which was significant ($p < .05$) and the Kaiser–Meyer–Olkin measure, which was .74 (.60 is considered as the minimum value for a good factor analysis[19].

PCA revealed the presence of three factors with eigenvalues exceeding 1, explaining 50.2, 20.6, and 11.1 % of the variance respectively. The scree plot also pointed to a three-factor solution. All three factors showed a number of strong loadings and all variables loading substantially on only one component. Table 7 shows the pattern and structure matrix for the solution. The first factor contains PPtol and the sensory and affective ratings of the discrete stimuli, named "Moderate-level stimulus appraisal & pain tolerance" for future reference. The second factor holds all three PPth measures and will be referred to as "Pain threshold", and as the third factor comprises the ratings of the stimulus at tolerance level this factor will be referred to as "Tolerance-level stimulus appraisal". Moderate correlations were found between several factors, indicating that the oblique rotation was suitable [19]: $R^{\text{Moderate-level stimulus appraisal \& pain tolerance, Pain threshold}} = -.36$, $R^{\text{Moderate-level stimulus appraisal \& pain tolerance, Tolerance-level stimulus appraisal}} = .33$ and $R^{\text{Pain threshold, Tolerance-level stimulus appraisal}} = .04$.

Table 6. Pearson correlations between pressure-pain measures.

	1. Mean PPth leg	2. Mean PPth Back	3. Mean PPth arm	4. PPtol	5. Sens. rating PPtol	6. Affect. rating PPtol	7. Sens. rating 294 kPa	8. Affect. rating 294 kPa	9. Sens. rating 490 kPa	10. Affect. rating 490 kPa
1.	-	.654	.712	.396	.088	-.111	-.239	-.227	-.139	-.220
2.	-	-	.731	.359	.069	-.146	-.375	-.399	-.285	-.345
3.	-	-	-	.442	.036	-.207	-.382	-.439	-.241	-.356
4.	-	-	-	-	-.084	-.118	-.654	-.616	-.596	-.552
5.	-	-	-	-	-	.616	.456	.361	.504	.337
6.	-	-	-	-	-	-	.438	.453	.395	.467
7.	-	-	-	-	-	-	-	.935	.847	.853
8.	-	-	-	-	-	-	-	-	.785	.902
9.	-	-	-	-	-	-	-	-	-	.865

Table 7. Pattern matrix of the principal component analysis with oblimin rotation. Only loadings >.72 are depicted.

	Moderate level stimulus appraisal & pain tolerance	Pain threshold	Tolerance-level stimulus appraisal
PPth leg		.913	
PPth back		.847	
PPth arm		.890	
PPtol	-.818		
Sensory rating 294 kPa	.903		
Affective rating 294 kPa	.871		
Sensory rating 490 kPa	.917		
Affective rating 490 kPa	.887		
Sensory rating PPtol			.894
Affective rating PPtol			.819

Discussion

The current study was designed to examine reliability, validity and interrelationships of several measures that can be obtained in experimental pressure-pain studies. Pressure-pain threshold was measured three times on six body points. Results of the current study demonstrated good test–retest reliability for the three repeated measurements, as indicated by inter-measurement correlations of .70 or higher. However, it was also found that every first trial on a body point yielded significantly higher thresholds as compared to the second and third trials, while no difference was found between thresholds for the second and third, indicating the necessity of a practice trial on every point that is measured. Thus, practicing threshold measurements on only one body point, as was done in the current study, is not enough. Based on these findings, it is recommended to exclude first measurements for *every* body point from further analyses. Also, since no differences were found between second and third measurements, only two consecutive measurements (in addition to the practice trial) are needed.

As threshold measures on different body parts are often combined into one mean-aggregated individual PPth, internal consistency of three regularly used combinations of separate PPths (i.e. bilateral, unilateral, all) was also investigated in the current study. Although all three combinations showed good internal consistency (i.e., Cronbach's alpha values > .80), consistency was highest for bilateral combinations

(combining PPth's of one body part bilaterally assessed, e.g., left and right arm). Thus, the correspondence between threshold-values of identical left versus right body parts is larger than the correspondence between different body parts. Although it can be concluded that mean-aggregations of all combinations of threshold are adequate, aggregation across different body parts will lead to less reliable measures as compared to bilateral same body parts. Thus, we advocate to aggregate bilateral combinations of pain threshold values.

The factor solution as assessed in the current study with principal components analysis shows a three-factor structure: *Moderate-level stimulus appraisal & pain tolerance*, *Pain threshold*, and *Tolerance-level stimulus appraisal*, with moderate correlations between factors. Based on this factor solution it is concluded that pressure-pain responsiveness indeed consists of different dimensions. Each dimension (i.e. factor) adds to the underlying construct. Studies that do not include all of these three distinct dimensions of pain may yield an incomplete picture of pain responsiveness.

In the three-factor solution, subjective ratings of moderate pain stimuli (i.e. 294 and 490 kPa) describe a different aspect of pain experience than threshold measures, and surprisingly the subjective ratings tap the same dimension as (maximum) pain tolerance (in kPa), whereas the subjective judgments of this maximal load forms a separate factor. These results may indicate that PPtol is a more subjective measure of pain responsiveness (sharing the same subjective judgment aspect with the judgment of lower intensity stimuli), while PPth measures may be considered as more objective measures of pain responsiveness. The subjective nature of PPtol is also demonstrated by the finding that the affective and sensory ratings are highly correlated at lower intensities (around .90) and at the tolerance intensity only a .60, implying that while at lower intensities sensory and affective aspects are not distinguished from each other, they are well distinguished at tolerance intensity. The high correspondence found between pain tolerance and subjective pain reports to moderate pain stimuli also implies that the tolerance for a hardly bearable painful stimulus may also be assessed by subjective reports of pain to much lighter pressure intensities, thereby avoiding discomfort in the subjects.

The strength of the relation found between affective and sensory ratings might be interpreted as that people can hardly discriminate subjectively between these two aspects of pain. There is, however, a solid physiological basis to consider affective and sensory ratings as different aspects of pain. The correspondence found in the current

study may, on the other hand, also be due to the fact that a common instrument was used, i.e. the visual analogue scale on which the subjects could rate the intensity and unpleasantness of the stimuli. This explanation is supported by the findings of other studies using numerical descriptor scales to assess unpleasantness and intensity of stimuli. These studies did find differences between unpleasantness and intensity ratings[1,5], indicating a role for scale properties.

The current study can contribute to understanding some inconsistencies among the results of previous pain studies. For example, the clear distinction we have found between first and subsequent measurements of pressure-pain thresholds could explain the differences between previous studies that focused on the relations between pain thresholds and clinical pain in fibromyalgia samples. Pressure-pain threshold was found to be related to pain experienced in the present *past* but not to *present* pain in some studies[10,20], while the opposite pattern was found in another study (i.e. a relation was found with *present* pain, but not with pain in the present *past*[11]. In the first two studies mentioned, however, threshold was measured with *one* trial, while in the last study threshold was averaged over three trials. The results of the current study suggest that threshold measures averaged over multiple trials are more accurate reflections of true pain responsiveness (i.e. sensory processes), and therefore they should more reliably correspond with present pain. It may be speculated that the results obtained from a single trial threshold are confounded by psychological mechanisms. This is supported by the finding that a one-trial threshold was related to retrospectively reported pain in the past. It is known that retrospective report of complaints (such as in report of pain in present past) yields higher complaint scores compared to momentary report[21]. This discrepancy between retrospective and momentary report is probably (just as the first threshold) also related to psychological factors[22]. Thus, the relation between single-trial thresholds and pain in present past (as has been found by Geisser et al. and Giesecke et al.[10,20] could possibly reflect some psychological factors, while the relation found between three-trial thresholds and present pain (as has been found by Lautenbacher et al.[11] reflects true pain responsiveness (i.e. sensory processes).

Several limitations of our study have to be mentioned. First, only females within a specific range of age participated in this study. This limits the generalizability of the results to males and older subjects. A recent review shows that females have lower pressure-pain threshold and tolerance levels compared to males[23]. Also, there is some evidence that emotional and cognitive processes affect pain measures more in males

than in females[24-26]. Thus, it is possible that somewhat different results will be found when the current study is replicated with a male sample. Specifically, we would expect that in males more practice trials are needed to get an accurate PPth measure by lowering anxiety levels. Concerning our recommendation to aggregate measures across bilateral body parts, we would expect this to be valid for males as well, as gender differences in pain measures are found on several different body parts[23]. Another limitation is that experimental pressure pain is often used in the assessment of (chronic) pain cohorts and it is not clear whether the results from the current study can be extended to these groups. However, the sample in our study did entail several participants with pain complaints and preliminary results showed no difference in pain measures between these participants and pain free participants, indicating that the results can possibly also be extended to pain groups. Third, several findings in our study may also be ascribed to methodological issues. The finding that ratings of unpleasantness were not distinguishable from ratings of painfulness can be explained by the similarity of the scales used. As mentioned above, several studies using different scales did find differences in outcome measures. Further, it is possible that the factor solution found in our study represents underlying methodological differences and similarities between measurements: both the PPtol and the discrete stimuli were applied on the thumb nail with the algometer placed in a casket, in sight of the subject and the subject sitting on a chair, while for measurement of PPth, the subject was lying face down on a massage table and could not see the hand-held algometer. Future studies in which the factor analysis will be expanded with subjective ratings of stimuli delivered in the same manner as PPth was assessed, would give further insight in this issue. Finally, a relatively fast incremental rate of the pressure was used in the current study (i.e. 98 kPa/second), possibly affecting the accuracy of the tests because of short response times for the subjects in cases of low pain thresholds. However, the test-retest reliability was high for all body points tested while the pressure pain thresholds differed, indicating that this was not the case.

Summarizing, the current study aimed to investigate reliability and validity of several pressure-pain methods. Despite some limitations, some recommendations for use of pressure-pain methods are formulated: first, PPth, subjective ratings of moderate intensity stimuli, and subjective ratings of the maximum intensity are distinct aspects of pain responsiveness. It is therefore recommended to include a measure of each of these three dimensions of pain when assessing pressure pain responsiveness.

Second, in the assessment of PPth, we recommend one practice trial per body point and two consecutive trials for computing a mean PPth per body point. Further, when it is desirable to collapse PPth on several body points into one mean PPth, we suggest to average over bilateral body points only.

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Chapter 4.

Can mental effort investment measured with pupillometry be used as an objective measure of cognitive fatigue?

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Abstract

Introduction: Cognitive fatigue is a common complaint in chronic fatigued patients. It is usually operationalized as subjective report which is sensitive to several biases and objective measures are lacking. Results from brain imaging studies suggest that the amount of mental effort investment increases during fatigue inducement, but interpretations of such studies are equivocal. Dilation of the eye pupil diameter may be a more feasible and direct reflection of mental effort. Two studies are presented that explore the usability of pupillary responses during cognitive performance as an objective measure of cognitive fatigue. ***Methods:*** In Study 1, *n* = 45 college students (73.3 % females) participated in a protocol in which fatigue was induced by repetition of a cognitive task. In Study 2, *n* = 18 female college students and staff participated in a protocol in which fatigue was induced by low-grade inflammation through vaccination. In both studies self-reported fatigue, pupillary responses to a cognitive task, and performance accuracy were assessed. ***Results:*** In Study 1, a significant increase in self-reported fatigue was found, but unexpectedly, performance accuracy was increased and pupillary responses were reduced after fatigue inducement. Self-reported fatigue was not related to pupillary responses at any time point, but it was inversely correlated to the performance after fatigue inducement. In Study 2, self-reported fatigue, performance accuracy, and pupillary responses did not change over the protocol, and no relations were found between these measures. ***Discussion:*** Self-reported fatigue could not be related to a change in eye pupil diameter (as potential objective measure of mental effort investment and cognitive fatigue) in either of the studies. Limitations of both studies regarding fatigue manipulation and pupil measurement are discussed.

Introduction

Fatigue is a multidimensional concept, encompassing both physical and cognitive (including motivational) components. A distinction is often made between peripheral fatigue ("the inability to sustain a specific force or work rate [that] is limited to exercise or physical activity"[1]) and central fatigue ("the failure to initiate and/or sustain attentional tasks and physical activities requiring self-motivation"[1]). Whereas physical fatigue can have both peripheral and central components, cognitive fatigue is considered to originate from central mechanisms[2]. Chronic fatigue is an important complaint in many medical diseases, but the experience of fatigue can often not completely be explained by disease activity[3]. Moreover, chronic fatigue can also be present without an apparent medical disorder[4]. Cognitive fatigue is a common complaint in patients experiencing chronic fatigue[5,6], possibly pointing to central mechanisms underlying the fatigue.

The measurement of fatigue is most often based on the subjective experience of fatigue (i.e. through self-reports). However, self-reports are subject to inter- and intra-individual differences in mood and emotional valence of the fatigue symptoms[7] and thus, to gain a more comprehensive take on fatigue, objective measures are necessary. Furthermore, whereas subjective self-reported measures provide us with insights in the experience of fatigue, objective measures will enable research on physiological mechanisms underlying fatigue.

Objective measures for physical fatigue are well described, but it remains unclear how to objectively measure cognitive fatigue[3]. In analogue to exercise or physical activity output as an objective measure for peripheral fatigue, performance on cognitive tasks is often used as an objective measure of cognitive fatigue. However, the relationship between subjectively reported fatigue and performance to cognitive tasks is not always apparent; several studies have shown that cognitive fatigue can be present without effects on cognitive performance[8,9]. This points to the role of (compensatory) cognitive effort to maintain the performance level despite growing fatigue.

A better objective measure of cognitive fatigue may be the amount of effort that is invested during cognitive performance in combination with performance level. Several functional brain imaging studies in chronic fatigued patients have shown differences in brain activity during cognitive performance (i.e. increased allocation of mental effort compared to non-fatigued individuals), and it has been suggested that these differences might indicate a role for the compensatory effort that is invested in the performance[10-12]. The same was suggested by Harrison et al.[13] who induced (self-

reported) cognitive fatigue by way of low-grade inflammation and reported subsequent heightened brain activity during cognitive performance without changes in performance. Thus, increased mental effort may explain why performance is not always affected by fatigue and it is possible that performance only decreases when effort allocation reaches a ceiling.

Results from brain imaging studies depend on the brain areas that were included in the analyses. Furthermore, interpretations are not unequivocal, and expensive devices are needed. A straightforward, less expensive general index of mental effort can be found in pupillometry, the measurement of the dilation of the eye pupil. The eye pupil diameter is controlled by two opposing muscles groups within the iris of the eye which in turn are controlled by the parasympathetic and the sympathetic nervous system. Dilation of the pupil diameter is a reflection of increased sympathetic and decreased parasympathetic activity and can be interpreted as the effort that is invested, with greater dilations indicating a greater amount of effort[14]. A vast amount of studies have shown pupillary dilations to be able to differentiate between task demands in several different cognitive paradigms[15,16] and between (clinical) groups[17].

The current paper presents two studies on the usability of pupillometry as an objective measure of mental effort investment in the framework of cognitive fatigue. First, within-subject changes were tested on self-reported momentary fatigue and task performance and pupil diameter on two cognitive tasks (i.e. Stroop and *n*-back). The tasks entailed different attention requirements and several difficulty levels. In the first study, fatigue was induced by repetition of a cognitive task (an extended *n*-back). In the second study, fatigue was induced by S*almonella typhi* vaccine as an immunological challenge. Vaccine-induced fatigue is an innovative experimental model for inflammation-related fatigue. It was expected that fatigue inducement would lead to increased self-reported fatigue and increased mental effort investment (i.e. a larger dilation of the eye pupil) without changes in performance. It was also expected that at more difficult task levels, effort would not further increase and performance would decrease. Second, between-subject relations in self-reported fatigue, task performance and pupil diameter were tested. It was expected that especially after fatigue inducement, subjects high on self-reported fatigue either would have a higher mental effort investment (i.e. a larger dilation of the eye pupil), or a lower effort investment together with performance decrement.

STUDY I

Methods

Participants

Participants were recruited from a participation list of an internet survey for undergraduate and post-graduate college students at Utrecht University. To ensure a wide range in fatigue, persons were selected based on their score on a fatigue questionnaire (CIS-20-R)[18] in the internet survey, including only persons with a relatively low score (< 62) or a relatively high score (> 81). Self-assessed fatigued subjects (*n* = 7) were additionally recruited through posters and flyers on campus. Retrospective fatigue scores obtained on the test-day (CIS-20-R) did not show two distinctive groups however, but rather a normal distribution in fatigue with a wide range in scores (M: 61.93, SE: 3.11, range: 30 - 101), and thus participants were treated as one group. Exclusion criteria for all participants were self-report of a current medical condition and use of prescribed medication. Eligible persons received either a monetary incentive or credits for participation.

Design and procedure

In a within-subjects design with (partly) repeated measures, performance and pupillary responses were assessed on two cognitive tasks (Stroop and *n*-back I) before and on one task (*n*-back II) after fatigue inducement. Presentation of an extended *n*-back task (during 25 minutes) was used to induce fatigue. Momentary fatigue and motivation were assessed immediately before task onset (after instructions). Self-reported invested effort was assessed after completion of each task. See Figure 1 for a schematic overview of the procedure.

Self-reported fatigue, motivation, and effort

Retrospective fatigue was assessed with the Checklist Individual Strength Revised (CIS-20-R)[18], a list comprising 20 items regarding fatigue experience in the past two weeks.

Subjective momentary fatigue was assessed with a visual analogue scale (“Indicate how tired you are feeling at this moment”; “not tired at all” – “completely exhausted”). Task-related momentary motivation to perform was also assessed with a visual analogue scale (“Indicate how much you feel like performing on the upcoming task”;

"not at all" – "very much"). Task-related momentary perceived effort was assessed with the Rating Scale Mental Effort[19].

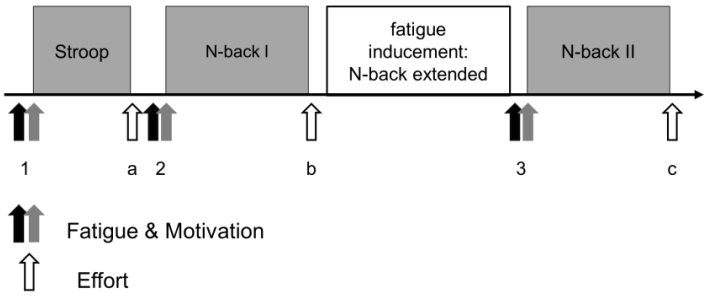

Figure 1. Schematic overview of task presentation and assessment of self-reported momentary fatigue and motivation (before each task onset, at measurement 1-3), and invested effort (after each task, at measurement a-c).

Cognitive tasks

In a classic Stroop task, a color word (i.e. red, green, blue, yellow) was presented in capital letters in either a font color congruent to the meaning of the word (e.g. the word RED, printed in red) or in an font color incongruent to the meaning of the word (e.g. the word RED, printed in blue). Thus, the task consisted of two levels: a congruent and an incongruent level. For every word, the color in which the word was printed had to be indicated as quickly as possible by pressing one of four buttons. The answers that corresponded to the buttons were presented in dark grey at the bottom of the screen to ensure eye fixation on the screen. Stimuli were presented for 2000 ms, followed by a crosshair during 2000 ms. Following instructions and 48 practice trials, 48 congruent and 24 incongruent trials were randomly presented in a single session with a short pause (300 ms) after 36 trials.

In the *n*-back task, single letters were presented one-by-one on the screen. Participants were instructed to indicate whether the letter on the screen was the same as the one *n* stimuli ago by pressing one of two buttons. The task included three difficulty levels: 1-back, 2-back, 3-back, and 4-back. Within each level, 21 target trials (i.e. requiring a "yes"-response) and 42 non-target trials (requiring a "no"-response) were presented. Levels were presented three times (per presentation 7 target trials; 14 non-target trials) in semi-random order with randomized stimulus presentation within

each level presentation. Each trial started with the presentation of a crosshair for 1500 ms followed by stimulus presentation for 2000 ms. Instructions for this task were given both orally and on the computer screen, followed by a practice round during which the instructor remained present to give further instructions if needed. Both tasks were presented with Presentation version 14.9 (Neurobehavioral Systems, Inc.) on a 24 inch computer screen.

Pupillary responses

The eye pupil diameter was recorded with a X60 Tobii eye tracking unit (Tobii Technology AB, Sweden) which was positioned under the screen on which the tasks were presented. Calibration of the eye tracker was performed before each task onset, leading to precise pupil diameter (mm) measurements. Sample frequency was 60 Hz. To minimize pupillary reactions to light changes, all stimuli were presented on the same background and font colors in the Stroop were equal in brightness. Pupil data was processed in LabWindows/CVI Version 9.1.0 (National Instruments Corporation, Austin, USA). Small gaps in the data were filled with interpolation after which the data was smoothed with a moving average algorithm. Trials with gaps > 200 ms or insufficient baseline data (less than five data points available) were manually detected and removed. Trial-by-trial pupillary responses were calculated as the change in pupil diameter between baseline (i.e. average diameter in 200 ms before stimulus onset) and the peak in pupil diameter between stimulus onset and response. Trials with pupillary responses < 0 were recorded as zero-responses. A mean pupillary response was calculated for each level of both tasks including only trials on which a correct response was given.

Performance accuracy

The hit rate (number of correct responses divided by the total number of trials) was computed for both levels of the Stroop and the sensitivity index (d' = Z(hit rate)-Z(false alarm rate; number of incorrect responses on non-target trials divided by total number of non-target trials) was computed for each level of the *n*-back. The sensitivity index indicates the ability to discriminate between targets and non-targets in the *n*-back. When all hits are made by chance d' is 0 and higher values of d' imply better ability to discriminate. To avoid proportions of 0 and 1 in calculation of d', hit rates of 1 were

adjusted to 1-1/2N and false alarm rates of 0 were adjusted to 1/2N, with N being the number of trials on which the proportion was based[20].

Analyses

Trials with reaction times faster than 150 ms were omitted. Mean pupillary responses within a task-level were considered missing when a pupillary response was available on less than 10 trials within that level (Stroop) or within the target or non-target trials of that level (*n*-back). Pupillary responses (all tasks), hitrates (Stroop task), and *d*' (*n*-back tasks) were severely skewed, and thus, non-parametric tests were reported for these variables.

Within-subject effects of the fatigue-inducing manipulation (extended *n*-back) were tested by comparing self-reports of fatigue, motivation and effort between the time points and by comparing performance and pupillary responses between the *n*-back I and the *n*-back II for every level of the task. Between-level differences in performance and pupillary responses within every task were also assessed. Relations between self-reported momentary and retrospective states of fatigue, effort and motivation, performance and pupillary responses were assessed at every time point. Alpha was set at .05 and corrected (Bonferroni method) for multiple comparisons where appropriate.

Results

Sample description

Forty-five persons participated (mean age: 23.73, SE: 0.78; 73.3% females). One subject was excluded from the Stroop because of incorrect responses to all incongruent trials, indicating that this subject had not understood the task. One subject decided to withdraw from the experiment after completing the *n*-back I. After applying the above described criteria for pupillary data, mean pupillary responses on the Stroop were available for $n = 41$ on the Stroop, $n = 41$ on the *n*-back I, and $n = 40$ (1-back) to 36 (4-back) on the *n*-back II. Momentary fatigue data was missing for one participant on time 2 (before onset of the *n*-back I). Motivation data was missing for one participant on time 1 (before onset of the Stroop) and for another participant on time 2 and 3 (before onset of the *n*-back I and before onset of the *n*-back II).

Effects of the extended *n*-back on self-reported momentary fatigue, motivation, and effort

Changes in the self-report measures are depicted in Figure 2. Momentary fatigue increased between time 2 (before *n*-back I) and time 3 (before *n*-back II) (F (2,40) = 35.57, $p < .001$; pairwise comparison time 2 and time 3: $p < .001$, other comparisons $p > .025$). Task repetition was thus successful in inducing fatigue. Motivation decreased over all time points (F (2,38) = 71.50, $p < .001$), all pairwise comparisons $p \leq .001$). Motivation was lower before on the *n*-back I compared to before the Stroop and further declined after the extended *n*-back, before the n back II. The reported invested effort after each task increased over time (F (2, 41) = 108.34, $p < .001$; all pair wise comparisons: $p < .001$). Reported effort was higher after the *n*-back I than after the Stroop and was further increased after the extended *n*-back.

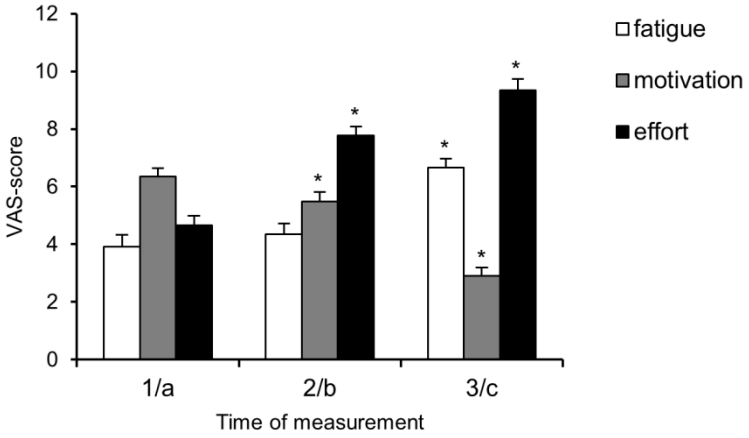

Figure 2. Changes in momentary fatigue, motivation, and effort during the protocol.
Notes. 1-3: fatigue and motivation measured directly before onset of each task; a-c: effort measured directly after each task). *: Significantly different from score on previous measurement.

Effects of the fatigue inducing extended *n*-back on performance and pupillary responses

Changes in performance accuracy and pupillary responses are depicted in figure 3. Comparisons of the *n*-back I and the *n*-back II for each of the four task-levels (i.e. 1-4 back) showed that the sensitivity index *d*' (performance accuracy) was higher in the *n*-back II as compared to the *n*-back I (Z = -2.63, p = .008) only for the 4-back, indicating

better instead of worse performance accuracy. For the easier levels no differences were observed. Pupillary responses were lower (i.e., the eye pupil diameter increased less) in the *n*-back II as compared to the *n*-back I (Z = -4.38, p < .001) only for the 1-back, indicating less instead of more effort investment. All other comparisons between the tasks were non-significant (p's > .0125).

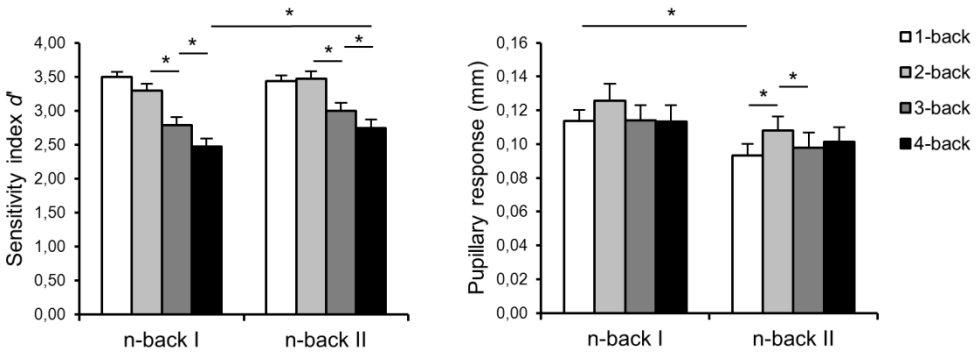

Figure 3. Sensitivity index and pupillary responses per level of the n-back I and n-back II. *: p < .05

Task-level differentiation by performance and pupillary responses

In the Stroop, the hit rate was lower and the pupillary responses were higher in the incongruent level compared to the congruent level (Z = -4.49, p < .001 and Z = -5.26, p < .001). Thus, performance accuracy and mental effort investment were influenced by the levels of the Stroop. In the *n*-back I, *d*' was decreased in the 3-back and the 4-back (χ^2 (3) = 48.34, p < .001; 2-back vs 3-back: p = .001; 3-back vs 4-back: p = .004) (see Figure 3), but pupillary responses did not differ between levels (χ^2 (3) = 5.58, p = .13). In the *n*-back II, *d*' was also decreased on the more difficult levels (χ^2 (3) = 28.79, p < .001, 2-back vs 3-back: p = .001, 3-back vs 4-back: p = .010), and pupillary responses were higher in the 2-back compared to the 1-back and lower in the 3-back compared to the 2-back (χ^2 (3) = 15.31, p = .002, 1-back vs 2-back: p = .002; 2-back vs 3-back: p = .003). Thus, performance accuracy was influenced by level difficulty in the *n*-back on both assessments. Pupillary responses were only influenced by the levels of the *n*-back after fatigue inducement.

Correlations between self-report measures, performance and pupillary responses

Retrospective fatigue (CIS-20R) was not related to any of the performance or pupillary measures in the Stroop or the *n*-back tasks, although a trend for a relation was found with *d'* on the 4-back of the *n*-back II (ρ = -.37, p = .018), pointing to decreased performance accuracy in persons with higher fatigue after fatigue inducement at the most difficult task level.

Momentary fatigue at time 3 was inversely related to *d'* in level 4 of the *n*-back II (ρ = -.45, p = .004). Self-reported effort at time 1 was inversely related to the hit rate in the incongruent condition of the Stroop (ρ = -.52, p < .001) and effort at time 3 was inversely related to *d'* in the 4-back level of the *n*-back II (ρ = -.52, p = .001). Higher levels of fatigue and effort were related to worse performance accuracy on the most difficult level of the *n*-back after fatigue inducement. Before fatigue inducement, higher effort was related to worse performance accuracy on the more difficult condition of the Stroop. The self-reported measures were not significantly related to pupillary responses at any time point.

Conclusion study I

In this sample with a wide range of fatigue, retrospective self-reported fatigue was not related to performance and pupillary responses on cognitive tasks. Momentary self-reported fatigue was related to performance accuracy on some time points, but not to pupillary responses. Although task repetition successfully increased self-reported momentary fatigue and effort and decreased self-reported motivation, the effects on performance accuracy and pupillary responses were minimal and unexpected: performance improved instead of reduced after task repetition, and the amount of eye pupil dilation (as a measure of effort investment) became smaller instead of larger after task repetition. These unexpected effects probably resulted from a practice effect. It may be speculated that this practice effect obscured the expected effects of an increase in cognitive fatigue. A different and stronger manipulation to induce fatigue may be needed to overrule practice effects. In Study 2, low-grade inflammation by vaccination is used as an adequate alternative method to induce fatigue. Experimentally induced low-grade inflammation by vaccination has been reported to

increase self-reported fatigue[13,21]. A placebo vs. vaccination design allowed to control for practice effects that seemed to play a role in the first study.

STUDY 2

Methods

Participants

Healthy non-smoking female participants were recruited among students and staff of the University of Birmingham by poster advertisement and word of mouth. Inclusion criteria were age between 18 and 50 years old and use of oral anti-conceptive medication. Persons were excluded in case of self-reports of a current medical condition and use of prescribed medication. Eligible persons received either a monetary incentive or credits for participation.

Design and procedure

In a placebo-controlled cross-over design subjects participated in two test-days, receiving a vaccination (*Salmonella typhi* capsular polysaccharide vaccine (0.025 mg in 0.5 ml, Typhim Vi, Sanofi Pasteur, UK) on one day and saline (placebo) on the other day. Order of vaccine/saline administration was counterbalanced across the participants. Dependent measures were taken on each test-day before injection and six hours post-injection.

The procedure is extensively explained elsewhere (Chapter 7, p. 119). In short, subjects were tested on two days which were similar to each other, with the exception of injection with either vaccine or saline. On a testing-day, several baseline measures were obtained (reported on elsewhere), including a blood sample upon arrival at the lab. Next, the subject was asked to participate in four cognitive tasks (of which two are reported on here) followed by an injection with either vaccine or saline. All measures were repeated six hours post-injection. Subjects were allowed to leave the laboratory in between test-sessions. The protocol was approved by the Health Research Authority NRES Committee West Midlands - South Birmingham.

Self-reported fatigue

Self-reported momentary fatigue was assessed with the Fatigue and the Confusion scale of a modified version of the Profile of Mood Scale[22] and the Motivation en Mental fatigue scale of the Multidimensional Fatigue Inventory[23]. Items were rephrased to represent momentary states and were to be answered on 5-point Likert scales. After recoding some of the items, higher sumscores represented higher levels of fatigue.

Cognitive tasks

Tasks were the same as in Study 1 (i.e. Stroop and *n*-back), with a few adjustments in the *n*-back to decrease the length of this task. The *n*-back task now included three levels (1-back to 3-back) with 15 target and 30 non-target trials per level. Levels were presented three times in a semi-random order with 15 trials (5 targets; 10 non-targets) per level presentation.

Pupillary responses and performance accuracy

The eye pupil diameter was recorded with an ASL H6 head mounted eye tracker (Applied Science Laboratories, Bedfort, USA). Output of this eye tracker is in pixels. As calibration of this eye tracker (which would give an estimate of pixel/mm ratio per assessment) is very time consuming and imprecise, pixel output was used for analyses. Trial-by-trial pupillary responses were processed in MatLab version R2012a (MathWorks, Inc., Massachusetts, USA). Data was interpolated and smoothed with a moving average algorithm as in Study 1. Because in Study 1, baselines obtained before stimulus onset often showed a negative slope (i.e. still recovering from the pupillary response on the prior trial), trial-baselines were now calculated as the mean over the first five data points (± 83 ms) after stimulus onset. Trial-by-trial pupillary responses were calculated as the change between baseline and the maximum pupil diameter between baseline and response. Trials were not manually inspected for gaps and missing values. Instead, trials with more than one missing value in the baseline or more than 30 % missing values in the time between baseline and response were deleted after processing in MatLab. Mean pupillary responses were further calculated as described in Study 1. Performance measures were the same as in Study 1.

Analyses

For both cognitive tasks, mean pupillary responses were considered missing within a level of a task when responses were available on less than 10 trials within that level (Stroop) or within the target or non-target trials of that level (*n*-back). Missing data were imputed with estimated means, using the mean pupillary responses at other time points as predictors. Results are provided for analyses on the raw data and in case of different results for raw and imputed data, both results are reported. A severe outlier (> 3 SD of mean) was detected in the mean pupillary response on the 3-back during the post-saline measurement. This response was excluded from all analyses regarding this measurement.

Within-subject effects of vaccination on self-reported fatigue, performance accuracy, and pupillary responses were tested as follows: Change variables (post-injection – pre-injection) were created within both conditions (i.e. saline and vaccination), resulting in Δ-saline and Δ-vaccine variables, which were then compared. In case of a significant difference between the change variables, post-injection measures were compared to pre-injection measures within each condition. As the change variables showed a normal distribution, paired samples t-tests were used for comparisons. Similar to Study 1, between-level differences in performance and pupillary responses within every task were assessed with non-parametric tests. Further, to test for a practice effect (that was suggested to account for findings in Study 1), pre-injection measures of pupillary responses and performance accuracy were compared to post-injection measures within the saline condition. Also, the first assessment of the protocol (pre-injection on first test-day) was compared to the last assessment (post-injection on second test-day). As in Study 1, between-subject relations between self-reported fatigue, performance and pupillary responses were also assessed. To account for multiple testing, alpha (initially set at .05) was adjusted according to Bonferroni method for all analyses.

Results

Sample description

Twenty-seven subjects were initially included in the study. We could however only use the data from 18 subjects because the vaccine batch that was used for the first 18 included subjects was recalled by the manufacturer and the new batch used for the

next nine subjects showed a completely different immunologic profile. Mean age of the remaining sample was 23.4 years (SE: 0.78, range: 19 - 30).

Based on the criterion for excluding mean pupillary responses, a number of mean responses were missing with a maximum of two missing values in the Stroop tasks (post-saline assessments) and three in the *n*-back tasks (pre-saline in the 1-back and post-saline in the 3-back). For one subject, one item was missing for the POMS fatigue scale and four items for the POMS confusion scale. Thus, the mean score of the fatigue scale was used for further analyses, and this subject was excluded from analyses on the confusion scale.

Effects of vaccination on self-reported momentary fatigue

The momentary self-reported fatigue measures (POMS-fatigue, POMS-confusion, MFI-motivation, MFI-mental fatigue) did not change between pre- and post-injection in either the saline or the vaccine condition (all *p*-values > .025) (see Figure 4). Likewise, post-injection fatigue or motivation was not different between the saline and the vaccine condition (all *p*-values > .05).

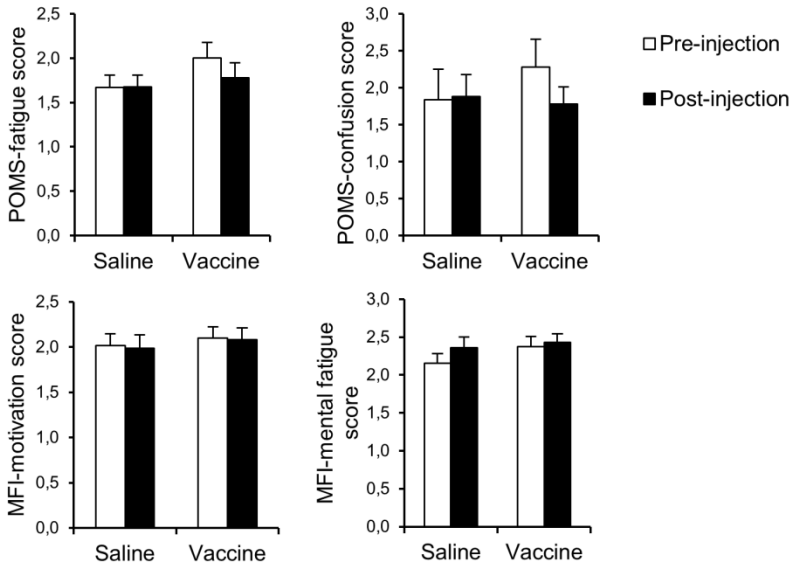

Figure 4. Mean scores on the four fatigue scales per measurement.

Effects of vaccination on performance and pupillary responses

Comparisons of Δ-saline and Δ-vaccine on hit rates in the Stroop and *d*' in the *n*-back showed no significant differences in any of the task levels (see Figure 5) The change in pupillary response was significant for the 2-back level of the *n*-back in the imputed data set (Z = -2.243, p = .025) but not in the raw data set (Z = -1.922, p = .055). Single comparisons on raw and imputed data did not show a difference in pupillary responses between pre- and post-injection in either the saline or the vaccine condition. No other significant differences between Δ-saline and Δ-vaccine were found.

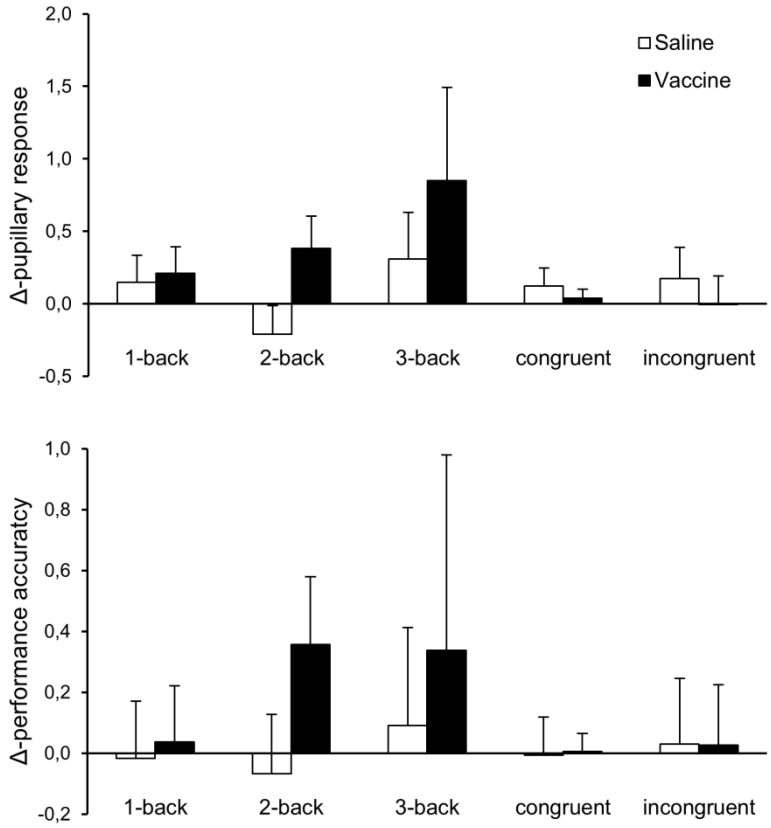

Figure 5. Change in pupillary responses and performance accuracy (d' and hitrate) between pre- and post-injection per separate level of the n-back (bars on the left) and the Stroop (bars on the right) for the saline and the vaccine condition.

Task-level differentiation by performance and pupillary responses

As in Study 1, hit rates and pupillary responses differentiated between the levels of the Stroop on all assessments (lowest test result hit rates: Z = -2.91, p = .004; pupillary responses: Z = -2.99, p = .003), with higher hit rates and lower pupillary responses in the congruent level. In the *n*-back, *d*' differentiated between the levels on all assessments (lowest test-result: χ^2 (2) = 6.70, p = .035), with a higher *d*' on the 2-back compared to the 3-back in all pre-injection measures and in the post-vaccine measure ($p \leq .005$). In the post-saline measure, *d*' was higher in the 3-back compared to the 1-back (p = .006). Pupillary responses did not differ between levels of the *n*-back on any of the assessments, concurrent with findings on pre-fatigue inducement in study 1.

Practice effects on performance and pupillary responses

Figure 5 shows a negative change score in pupillary responses for the 2-back level of the n-back, indicating reduced effort on the post-injection measure, and a positive change score in performance accuracy for the 3-back level, indicating increased performance in the post-injection measure. Comparisons of pre- and post-injection measures were non-significant, however (Z = -1.48, p = .14; Z = -0.21, p = .84).

Comparisons of performance and pupillary responses between the first assessment and the last assessment within the protocol showed lower pupillary responses for the 2-back level of the *n*-back (Z = -2.48, p = .013) and higher *d*' on the 3-back (Z = -3.10, p = .002) on the fourth assessment, indicating less effort and higher performance accuracy on the last assessment in the protocol.

Correlations between self-reported fatigue, performance and pupillary responses

Only self-reported momentary fatigue (POMS fatigue scale) was inversely related to *d*' on the 1-back of the post-vaccine measure (ρ = -.62, p = .006). No other significant relations were found.

Conclusion Study II

As reported elsewhere, our vaccination manipulation led to low-grade inflammation (Chapter 7, p. 123), with a 3.5 fold increase in the inflammatory marker interleukin-6 in the vaccine condition, but not in the saline condition. However, the current results show

that despite this effect, no increase is found in subjective fatigue after vaccination. Additionally, vaccination did neither influence performance accuracy nor pupillary responses.

General discussion

Both studies failed to support the usability of a change in eye pupil diameter (a measure believed to correspond with a change in mental effort investment) as a possible objective measure of cognitive fatigue. These studies however, also demonstrate how difficult it is to experimentally induce cognitive fatigue and reliably assess pupillary responses. In the first study performing on an extended cognitive task led to the expected increase in self-reported fatigue, but performance accuracy and pupillary responses changed towards a *less* fatigued pattern pointing to a practice effect: subjects performed better and invested less effort. This practice effect was replicated in the second study, emphasizing that the changes found in the first study were probably not due to increased fatigue. In the second study, it was shown that a vaccination producing low-grade inflammation did not affect subjective fatigue and it also did not influence task performance accuracy or pupil diameter. In both studies, between-subject correlational analyses pointed to the same direction: pupillary responses were not related to any of the self-reported subjective measures, while performance measures to some extent were. Our results correspond with the literature where relations between subjective fatigue and objective measures of performance or mental effort (brain activity) are sometimes reported[12,13], but not always[24]. Finding such an effect may very well depend on the manipulations of (cognitive) fatigue and the assessment of mental effort investment by pupillometry as explained below.

Manipulations of fatigue

The first manipulation to induce fatigue (used in the first study) was a presentation of an extended version of the same cognitive task that was also used to assess performance and mental effort. Although this manipulation was successful in increasing self-reported fatigue, there are some downsides too. First, as discussed above, repeated exposure to the same kind of task might have led to a practice effect, obscuring possible effects of the fatigue inducement. Second, participants stated that performing on the same task for such a long time was perceived as very boring. This was also reflected in decreases

in self-reported motivation. This might have affected the performance and invested effort on the last task. On the other hand, self-reported effort was highest for the last task indicating that despite decreased motivation, participants still aimed to perform well on the task. Also, it is not clear whether presenting a different cognitive task to induce fatigue would have been less boring.

The second manipulation to induce fatigue (used in the second study) was administration of a vaccination, leading to low-grade inflammation. Contrary to other reports[13,21] the vaccination did not lead to increases in subjective fatigue in our study. One major difference between the current study and the literature is that solely women were included in the current study whereas earlier reports included only men. Animal models show some gender differences in behavioral responses to inflammation[25,26] and it might be possible that in humans, gender differences also exist in responses to inflammation. Furthermore, the relative small sample size of the second study has limited the statistical power resulting in the ability to demonstrate only large effects.

Use of an eye tracker as measure of mental effort investment

Accuracy of the measurement of the pupil diameter depends on the hardware used. In the first study, an eye tracker unit was used that required the subject to remain in a fixed position during the measurements. A hidden camera revealed that many subjects were unable to retain this position which resulted in data loss. In the second study, this problem was solved by using a head-mounted eye tracker and indeed, less data was lost. However, this particular unit disabled adequate calibration and thus amplified the noise between the repeated measurements. It is very well possible that the pupillary measurements would be more sensitive when an (head-mounted) eye tracker is used that produces more accurate readings.

Conclusion

Neither of the studies presented in the current paper support the idea that pupillary responses that are believed to be a measure of mental effort could be used as an objective measure of cognitive fatigue. However, important limitations of these studies in fatigue inducement and eye tracker hardware might account for the lack of findings and thus, additional studies on the role of pupillary responses in cognitive fatigue assessment are required.

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Chapter 5.

Biological and psychological predictors of visceral pain sensitivity in healthy premenopausal women

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Abstract

Background: Factors that are associated with pain perception remain incompletely understood, especially in the visceral pain field. Therefore, the current study aimed to investigate possible psychological and biological predictors of visceral pain sensitivity in healthy subjects. ***Methods:*** In a sample of 59 healthy premenopausal female subjects on hormonal contraceptives, measures of gastrointestinal (GI) symptoms in daily life, trait and state anxiety, depression, serum cortisol concentrations, and serum levels of interleukin-6 (IL-6) were obtained, followed by assessment of rectal distension pain sensitivity measures (i.e. rectal distension sensory threshold, pain threshold, and pain ratings for discrete rectal distension stimuli). ***Results:*** Regression analyses showed that more GI symptoms in daily life predicted a lower pain threshold. Higher levels of state anxiety predicted a lower pain threshold. Higher cortisol concentrations predicted lower pain ratings. IL-6 was positively related to GI symptoms but was a non-significant predictor of pain threshold in the multiple regression analysis. ***Conclusions:*** Similar to findings in patients with functional GI symptoms, we showed that sub-clinical GI symptoms predict visceral pain sensitivity. In line with somatic pain findings, state but not trait anxiety was found to predict visceral pain sensitivity. Our finding on serum cortisol as positive predictor of pain sensitivity might be interpreted in light of immunosuppressive effects of cortisol. Our finding on the role of IL-6 in GI symptoms is promising for understanding gastrointestinal complaints in patients and needs further investigation.

Introduction

While pain is a common symptom in many medical conditions, understanding the perceived intensity or duration of pain is often more complicated than the medical condition as such. Furthermore, symptoms of pain can be present even in the absence of a detectable pathological origin. Although pain sensitivity has been studied extensively both in clinical and experimental pain paradigms, the mechanism(s) mediating pain sensitivity remain incompletely understood but almost certainly encompass psychological and biological factors. One step toward understanding *increased* pain sensitivity, or hyperalgesia, in patients is to disentangle the mechanisms involved in the substantial inter-individual variations in pain sensitivity that exist not only in patient populations but also in healthy subjects. To this end, we herein present a hypothesis-driven approach to disentangle the contributions of a range of psychological and biological variables to inter-individual differences in visceral pain sensitivity in healthy subjects. Increased visceral pain sensitivity has been demonstrated in patients with irritable bowel syndrome (IBS)[1,2], the most common functional gastrointestinal disorder. Therefore, studies addressing predictors of visceral pain are highly clinically relevant.

First, gastrointestinal symptoms experienced in daily life was expected to predict visceral pain sensitivity. Severity of gastrointestinal symptoms is reportedly positively associated with visceral pain sensitivity in functional abdominal pain[3,4]. Thus, we expected that individual differences in gastrointestinal complaints, albeit in a subclinical intensity, would predict rectal pain thresholds in a healthy sample as well, with more gastrointestinal complaints predicting lower pain thresholds.

Second, psychological factors such as anxiety and depression have frequently been put forward as important mechanisms of pain sensitivity. Although a relation between anxiety and experimental peripheral pain sensitivity has previously been demonstrated, findings for visceral pain sensitivity are inconsistent[2,5-7]. These inconsistencies might be explained by differential effects of trait versus state anxiety[8,9]. Previous studies focusing on the relation between depression and pain sensitivity also showed mixed results[1,7,10]. We expected trait scores for anxiety and depression to have a small positive effect on pain sensitivity, while the predictive value of state anxiety will be more substantial.

Finally, given previous evidence linking the hypothalamus-pituitary-adrenal (HPA)-axis and the peripheral immune system with peripheral and visceral pain sensitivity[11-13], we included serum cortisol and circulating levels of the pro-inflammatory cytokine

interleukin-6 (IL-6) as biological predictors. IL-6 has been found to induce allodynia in rodents[14,15] and a relation between IL-6 and visceral pain sensitivity has been reported in humans as well[16]. For a more complete assessment of pain sensitivity, we not only measured rectal sensory and pain thresholds but also responses to discrete rectal distension stimuli at the individual's pain threshold.

Methods

Participants

The sample consisted of N = 62 healthy female subjects recruited by local advertisement at the University Hospital of Essen and the surrounding universities in Essen, Duisburg, Düsseldorf and Bochum in Germany. Females were chosen given the well-known female preponderance of functional gastrointestinal disorders including IBS[17]. All participants were naïve to balloon distensions studies. Exclusion criteria included age <18 years and >45 years, body mass index < 18 or ≥ 30, any concurrent medical condition, including gastrointestinal, neurological, psychiatric, cardiovascular, immunological, and endocrine conditions. Any chronic medication use was also exclusionary, except hormonal contraceptives (see below), thyroid medications or occasional use of over-the-counter allergy or pain medications for benign headaches, cramps, etc. To exclude any (functional) gastrointestinal conditions, subjects were interviewed in detail about their medical history and specifically about relevant gastrointestinal symptoms and/or previous medical consultations due to gastrointestinal complaints. This was accomplished initially during a telephone screening and then in more detail during a personal interview which included a standard medical examination by a physician (author E.C.). In addition, subjects completed a standardized questionnaire assessing a variety of upper and lower gastrointestinal symptoms (for details, see section "questionnaires"). Sum scores ≥ 10 would have led to exclusion from the study – but this was not applied since the highest sum score in the present sample was 9 (see Table 1). A detailed assessment of lifetime history of gastroenteritis was not accomplished.

Only females on hormonal contraceptives were studied to reduce potential confounding by menstrual cycle phase. Information about the type of hormonal contraceptive (i.e., biphasic or triphasic) was however not recorded for all participants. All participants were evaluated digitally by a physician (author E.C.) for anal tissue

damage (e.g., painful haemorrhoids) which may interfere with balloon placement. A history of psychological conditions (based on self-report) or presently increased scores (i.e., scores ≥ 11) on the Hospital Anxiety and Depression Inventory (HADS) were also exclusionary[18]. The study protocol was approved by the local Ethics Committee (protocol number 08-3823). All participants gave written informed consent, and were paid for their participation.

The current analysis was conducted on baseline pain assessments from N = 47 healthy women who participated in a previously published study on visceral placebo and nocebo responses[19]. Experimental manipulations to induce placebo/nocebo expectations were conducted following these baseline assessments which are not reported on in detail in the published report[19]. An additional N = 15 women were recruited to enlarge the sample size for the current analysis.

Study design & procedures

During a prior appointment, informed consent was obtained and all trait variables were assessed. See Figure 1 for a schematic overview of procedures during the test-day. All measurements started between 3 and 5 pm to standardize possible circadian effects. Subjects were instructed to self-apply a commercially-available clyster (Clyssie, 120ml, Braun, Melsungen, Germany) two hours prior to arriving at the laboratory. The clyster was provided to volunteers together with written instructions during the initial appointment. Upon arrival to the laboratory, an indwelling intravenous catheter was placed into the non-dominant arm vein. After a resting period of 10 minutes, the baseline blood sample was collected together with the assessment of state anxiety using a validated questionnaire. Next, an infinitely compliant balloon was inserted into the rectum 5 cm from the anal verge. This balloon is a catheter-affixed polyethylene bag of cylindrical shape and 10 cm in length. Fully inflated it has a diameter of 8 cm and a maximum volume of 500 mL. Following balloon placement, another resting period of ten minutes was accomplished. Subsequently, sensory and pain sensitivity were measured, followed by another assessment of state anxiety. After this, four distinct phasic pressure stimuli were applied at the individuals' pain threshold level. A final blood sample was then obtained together with the assessment of state anxiety.

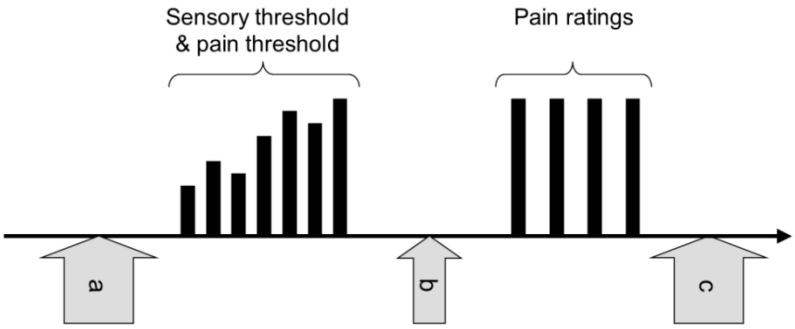

Figure 1. Schematic overview of the test-procedures, with predictor measurements at time point "a" (blood sample, state anxiety I); "b" (state anxiety II); and "c" (blood sample, state anxiety).

Pain sensitivity measures

Rectal distension sensory threshold, pain threshold, and pain ratings of discrete rectal distension stimuli were assessed as measures of visceral pain sensitivity. For assessment of the rectal sensory and pain thresholds, phasic balloon inflations with random increments of 2-10 mmHg were carried out with a pressure-controlled barostat system (modified ISOBAR 3 device, G & J Electronics, Ontario, Canada) using a double-random staircase protocol, as previously described[19,20]. Each pressure was maintained for 30 s, after which the subject was prompted to rate the sensation on a 6 point scale (i.e. 1 = no perception; 2 = doubtful perception; 3 = sure perception; 4 = distension, not unpleasant; 5 = distension, unpleasant; 6 = distension, very unpleasant/painful). The scale with both the numbers and written descriptors was printed on a sheet of paper which was kept clearly visible to volunteers during distensions. At the end of each distension, subjects were asked by the investigator to specify the rating orally. In-between individual distensions, the barostat was deflated to a pressure of 0 mmHg. The sensory threshold was defined as the first pressure at which the subject indicated a three on the scale (i.e. "sure perception") and the pain threshold as the first pressure at which the subject indicated a six (i.e. "very unpleasant or painful"). If the pain threshold was not reached at 50 mmHg, the procedure was stopped and 50 mmHg was recorded as pain threshold.

Subsequently, pain ratings were obtained for four phasic rectal distensions delivered 2 mmHg below the individuals' pain threshold level. Each distension was maintained for 30 s, after which the participant was prompted to rate the intensity of perceived pain on

a visual analogue scale ranging from 0 (no pain) to 100 (very much). The mean of the four ratings was used as pain rating.

Questionnaire variables

Subclinical gastrointestinal (GI) complaints during the preceding month were assessed with a standardized in-house questionnaire assessing frequency and severity of various relevant gastrointestinal symptoms (i.e., diarrhoea, constipation, vomiting, nausea, lower abdominal pain, upper abdominal pain, heart burn, postprandial fullness, bloating, loss of appetite) on a Likert-type rating scale (0 = experience never, 1 = experience once or twice per month, 2 = experience once or twice per week, to 3 = experience more than twice per week). For analysis, a sum score was computed. Trait and state anxiety were measured with the State-Trait Anxiety Inventory (STAI)[21]. Symptoms of depression was assessed with the Beck Depression Inventory[22].

Biological measures

Blood samples were analyzed for interleukin-6 (IL-6) and cortisol concentrations as follows. Plasma was obtained by centrifugation of EDTA-treated blood for 10 min (2000 xg) and was stored at -80°C until analysis. Plasma IL-6 concentrations were quantified using a bead-based assay (Bio-Plex Cytokine Assay, Bio-Rad Laboratories GmbH, Munich, Germany). Samples were prepared according to the manufacturer's instructions and were analyzed on a triple-laser FACSCanto II flow cytometer using FACSDiva software (BD Immunocytometry Systems, Heidelberg, Germany). Absolute cytokine levels were calculated based on the mean fluorescence intensity of cytokine standard dilutions with a 4 Parameter Logistics (4PL) curve model using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA). The detection limit of the assay was 0.2 pg/ml. Plasma levels of cortisol were determined using enzyme-linked immunosorbent assays (ELISA; IBL International, Hamburg, Germany) according to the test protocol of the manufacturer, and were analyzed on a Fluostar OPTIMA Microplate Reader (BMG Labtech, Offenbach, Germany). The detection limit was 0.83 nmol/l.

Statistical analyses

Analyses were performed with IBM SPSS for Windows, version 20. Missing values were imputed by expectation maximization method (for gastrointestinal symptoms and

for the psychological measures) or by the series means (for biological measures). See results section for more imputation information. All variables were checked for outliers and normality. Scores on the depression scale were severely skewed, which was not improved by log-transformation. Thus, this variable was retained untransformed and further analyses were done with nonparametric tests. Cortisol was square root transformed which reduced the number of outliers and improved the normality. IL-6 was inverse transformed, after which no outliers were detected and normality was improved. The inversed IL-6 data was multiplied by -1 to obtain scores that reflect the same direction as the untransformed data. Gastrointestinal complaints was square root transformed.

Associations between predictor variables and the pain sensitivity variables were assessed with Pearson's *r* or Spearman`s rho where appropriate. Associations with $r \geq .20$ (small to large effect sizes[23]) were included in (multiple) regression models for the sensory threshold, pain threshold, and mean pain rating. Multicollinearity was checked for all multiple regression analyses. Two-tailed *p*-values < .05 were considered significant.

Results

Sample description

Three subjects were excluded from analyses because of failures in measurement (n = 1) or high levels of IL-6 indicating disease at the time of the measurement (n = 2), resulting in a final sample of 59 subjects with a mean age of 24.8 years (SE = .66). Despite initial screening, two subjects in the sample had a score >18 on the BDI, suggesting clinical depression. As analyses excluding these subjects did not yield results different from analyses on all subjects, they were retained in the results. No participant had ever sought medical attention due to gastrointestinal symptoms (based on self-report).

Data description

See Table 1 for information on all variables. Individual data was missing for GI symptoms (*n* = 2), depression (*n* = 1), state anxiety (*n* = 1), cortisol (*n* = 1) and interleukin-6 (IL-6) (*n* = 1) and was imputed. Serum cortisol concentrations (t (58) = 4.528, $p < .001$) and state anxiety levels (t (58) = 2.679, $p = .01$) were significantly

higher before (*a*-variables in Table 1) as compared to after (*c*-variables in Table 1) the rectal distension protocol, indicating an effect of anticipation on these variables.

Table 1. Mean (SE), range, and skewness and kurtosis statistics of all untransformed variables before imputation of missing values.

	Mean (SE)	Range	Skewness (SE)	Kurtosis (SE)
Sensory threshold (mmHg)	15.46 (0.61)	4 - 28	0.14 (.31)	0.47 (.61)
Pain threshold (mmHg)	36.53 (1.26)	16 - 50	-0.11 (.31)	-1.24 (.61)
Mean Pain rating	51.00 (3.91)	0 - 95.25	-0.20 (.31)	-1.29 (.61)
GI symptoms	2.91 (0.33)	0 - 9	0.87 (.32)	0.29 (.62)
Trait anxiety	34.00 (0.89)	24 - 50	0.55 (.31)	-0.35 (.61)
Depression	4.45 (0.76)	0 - 28	2.13 (.31)	5.33 (.62)
State anxiety *a*	34.28 (0.88)	21 - 57	0.52 (.31)	1.22 (.62)
State anxiety *b*	32.88 (0.85)	20 - 46	0.09 (.31)	-0.61 (.62)
State anxiety *c*[a]	32.78 (0.88)	20 - 46	-0.15 (.31)	-0.44 (.62)
Interleukin-6 (pg/ml)	1.30 (0.21)	0.19 - 8.93	2.79 (.31)	9.10 (.62)
Cortisol *a* (nmol/l)	327.95 (18.82)	68.1 - 863.9	0.91 (.31)	2.05 (.62)
Cortisol *c*[a] (nmol/l)	272.77 (16.73)	90 - 698.3	1.23 (.31)	1.45 (.62)

[a] Measures of state anxiety and serum cortisol concentrations after assessment of pain sensitivity are depicted for a complete overview of obtained variables but were not included in the predictor analyses.

Predictors of visceral pain measures

Correlational analyses

Table 2 shows correlations between all variables. The sensory threshold and pain threshold were significantly correlated ($p < .001$), while mean pain rating was not related to either the sensory threshold ($p = .50$) or the pain threshold ($p = .48$). More gastrointestinal (GI) symptoms were related to a lower pain threshold. Higher State anxiety a was related to a lower pain threshold. A higher cortisol level was associated with a lower mean pain rating.

Regression analyses

Regression analyses were performed for the rectal distension sensory threshold, pain threshold and mean pain rating of distensions at pain threshold level (Table 3). For the sensory threshold, only one predictor (GI symptoms) could be identified with a correlation ≥.20. Although a regression model with one predictor would yield the same result as the correlational analysis reported on above, the model is still reported here

for readability purposes. The model with GI symptoms was not significant (adj R^2 = .03, F (1,57) = 2.98, p = .09). GI symptoms, state anxiety *a*, IL-6, and cortisol were entered in a multiple regression analysis with pain threshold as the dependent variable. The model explained 22% of the variance in pain threshold (adj R^2 = .22, F (4,54) = 5.012, p = .002). GI symptoms and state anxiety *a* contributed to the model, but IL-6 and cortisol did not. Cortisol and state anxiety *b* were entered in a model to predict the mean pain rating. The model explained 10% of the variance in the mean pain rating (adj. R^2 = .10, F (2,56) = 4.07, p = .02). Cortisol contributed significantly to the model while the contribution of state anxiety was not significant.

Thus, GI symptoms predicted the pain threshold with more GI symptoms being related to lower pain thresholds. Increased state anxiety levels also predicted a lower pain threshold. Regarding the biological measures, higher serum cortisol concentrations predicted lower pain ratings. IL-6 was not related to any of the pain measures.

Table 2. Pearson's *r* correlations for the relation between predictor variables and pain sensitivity measures in the gray area and relations between predictor variables in the white area.

	1. Sens thr	2. Pain thr	3. Mean Pain rating	4. GI sympt	5. Trait anx	6. Depr [a]	7. State anx *a*	8. State anx *b*	9. IL-6	10. Cort
1.	-	.45[d]	.00	-.22	-.12	-.03	-.17	-	-.13	.06
2.	-	-	- .01	-.33[b]	-.19	-.03	-.35[c]	-	-.23	-.21
3.	-	-	-	.13	.12	-.09	-	.20	-.02	-.29[b]
4.	-	-	-	-	.32[b]	.25[b]	.07	.15	.26[b]	-.04
5.	-	-	-	-	-	.55[d]	.36[b]	.41[b]	.07	-.02
6.	-	-	-	-	-	-	.27[b]	.34[b]	.10	.01
7.	-	-	-	-	-	-	-	.82[d]	.17	-.01
8.	-	-	-	-	-	-	-	-	.18	.02
9.	-	-	-	-	-	-	-	-	-	.05

Notes. Sen thr = sensory threshold; Depr = depression; Anx = anxiety; Cort = cortisol.

[a] For the correlations with depression, Spearman's rho is depicted; [b] Two-tailed p < .05;

[c] Two-tailed p < .01; [d] Two-tailed p < .001

Table 3. Results of (multiple) regression analyses.

Dependent variable	Predictor variables	*β*	*t*	*P*
Sensory threshold	Constant		9.59	<.001
	GI symptoms	-.22	-1.73	.09
Pain threshold	Constant		7.46	<.001
	GI symptoms	-.29	-2.38	.02
	State anxiety *a*	-.31	-2.62	.01
	IL-6	-.10	-0.81	.42
	Cortisol	-.22	-1.91	.06
Mean pain rating	Constant		3.02	.03
	Cortisol	-.29	-2.35	.02
	State anxiety *b*	.21	1.66	.10

Discussion

The current study aimed to assess several psychological and biological predictors of visceral pain sensitivity in a healthy female sample. We observed gastrointestinal (GI) symptoms, state anxiety, and serum cortisol concentrations to moderately predict (aspects of) visceral pain sensitivity. Trait anxiety and depression, however, appear to be unrelated to visceral pain sensitivity measures. Interleukin-6 was also not related to the pain sensitivity measures.

A relation between GI symptoms and pain sensitivity has previously been reported in IBS patients[4]. In line with our expectations, visceral pain sensitivity turns out to be related to GI symptoms in a healthy population as well (i.e. a population with only subclinical GI symptoms). Finding this relation in healthy subjects as well may be of relevance for future studies focusing on the role of visceral sensitization in the development of IBS. The relation between GI symptoms and IL-6 suggests that the reported GI symptoms were (partly) inflammation-related. Inflammatory mediators affect pain sensitivity through their effects on nociceptive neurons[24] and as such might explain the relation between GI symptoms and visceral pain sensitivity. However, we did not find support for a relation between IL-6 and visceral pain sensitivity. It is possible that the statistical power of our study was too low to demonstrate an effect of IL-6 in a healthy population as IL-6 levels would be relatively low. Local (i.e. in the gastrointestinal tract) inflammatory markers might have shown a stronger relation with visceral pain sensitivity.

It was expected that anxiety would predict visceral pain sensitivity. In line with our expectations, state anxiety indeed predicted the visceral pain threshold. Unexpectedly, trait anxiety did not. The differential effects of trait and state anxiety on pain sensitivity have been reported before in peripheral pain studies[9], and the current study adds to this that the same distinction can be found in a visceral pain paradigm. A positive relation between visceral pain sensitivity measures and state anxiety has been found in some previous studies as well[2], but not in all[5]. The latter study specifically induced anxiety prior to pain sensitivity assessment (as opposed to the studies finding a relation between state anxiety and pain sensitivity), possibly explaining differences in findings. Neuroimaging studies have shown negative emotions to affect neural processing of visceral pain stimuli in healthy persons, possibly explaining the relation between anxiety and pain sensitivity[25-27].

Although it was expected that trait depression would predict visceral pain sensitivity, we did not find such a relation with either of the pain sensitivity measures. However, depression scores in our healthy sample were low for the majority of subjects (as was illustrated by the severely skewed distribution of the scores) and probably too low to demonstrate a relation. It cannot be excluded that depression plays a role in visceral pain sensitivity since a relation between depressive mood (i.e. experimentally-induced as a state) and peripheral pain sensitivity has been reported in a healthy sample[28]. Another observation is that depressive symptoms are generally higher in patient groups with functional gastrointestinal symptoms. It remains unclear whether these symptoms affect their visceral pain sensitivity[1,4]. More research is needed to pinpoint a role of trait depression in visceral pain sensitivity and IBS.

Higher serum cortisol concentrations before visceral pain sensitivity measurements were found to be related to lower pain ratings (indicating decreased pain sensitivity). This relation has also been reported for peripheral pain (although there the relation was only found in men and not women)[29]. Congruently, experimentally induced hypocortisolism was shown to be related to increased pain sensitivity in healthy human subjects[30]. The immunosuppressive effects of cortisol[31] might explain the effects of cortisol on pain sensitivity. Inflammatory markers increase pain sensitivity and cortisol might inhibit these effects by suppressing the inflammatory response.

Limitations and recommendations

Despite inclusion of a relatively large sample in comparison to other pain studies, multiple regression analyses generally require even larger samples. Thus, our findings should be interpreted with caution and need replication. Our predictor variables explained up to 22 % of variance in the pain sensitivity measures, indicating that pain sensitivity is a complex phenomenon and that other predictors should be investigated as well. As we report on findings in premenopausal, healthy females on hormonal contraceptives, as a next step, results of this study should be replicated in samples differing in age, sex and hormonal status, as well as in patient samples. Both state anxiety and serum cortisol concentrations were significantly elevated prior to the visceral pain measurements, probably indicating anticipation of the upcoming protocol. Though we intended to measure the effect of a baselevel condition, it may rather be that stress-induced levels resulted in the predictions as observed. Future studies should take into account that base levels might be affected by anticipation of the pain measurement protocol. Finally, we acknowledge that other pro-inflammatory cytokines have been associated with pain sensitivity in animal models[32]. Assessing an array of cytokines, including both pro- and anti-inflammatory cytokines, would have clearly given a more thorough picture regarding circulating immune factors that may be associated with visceral pain sensitivity in humans. However, considering the exploratory nature of the current study, including more cytokine measures was out of our scope.

Conclusion

The current study shows that in a healthy female sample, subclinical gastrointestinal symptoms, state anxiety, and serum cortisol concentrations predict (albeit to a limited extent) different aspects of visceral pain sensitivity. Our findings confirm earlier findings on a difference between state and trait anxiety, with state, but not trait anxiety affecting pain measures. Our finding that state anxiety and cortisol were higher before compared to after the pain measurements suggests that anticipation of visceral pain measurements can influence findings on pain sensitivity, a notion that should be taken into account when interpreting pain sensitivity findings in patient groups.

Acknowledgments

We thank Mareike Bäsler, Julia Schmid and Kristina Bäumker for their excellent support during data acquisition.

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Chapter 6.

Infection load as predisposing factor for somatoform disorders: Evidence from a Dutch GP registry

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Psychosomatic Medicine, *in press*

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Abstract

Objective: Somatoform disorders are characterized by chronic multiple functional somatic (FS) symptoms. It has been suggested that infections may be triggers for FS symptoms to occur, pointing to the immune system as a pathogenic factor in their development. The current study aimed to compare the prevalence of infections (i.e. infection load) in the history of patients with somatoform disorders with that of matched controls. ***Methods:*** Samples (*n* = 185) were identified in the Psychiatric Case Register Middle Netherlands (PCR-MN) and the Julius General Practitioners Network (JGPN). Patients with a somatoform disorder diagnosis in the PCR-MN (SD patients) were compared to matched persons without somatoform complaints (Controls) on their infection load in two time periods *before* date of the psychiatric diagnosis or a matched date for the Controls (i.e. the total time period for which data were available and a three-year time period). Infection load was defined as the total number of infections documented in the JGPN. ***Results:*** SD patients had significantly more infections than Controls in both time periods (total time period: 0.87 ± 0.10 vs. 0.51 ± 0.06, $z = -3.08$, $p = .002$; three-year time period: 3.44 ± 0.47 vs. 2.15 ± 0.50, $z = -2.91$, $p = .004$).

Conclusions: Results show that SD patients have a higher infection load preceding their diagnosis as compared to matched controls, implicating that infection load may indeed predispose for developing FS symptoms. These findings emphasize the importance of further research on immunological mechanisms in FS symptoms. Limitations of the study are discussed.

Introduction

When a person presents with somatic symptoms that cannot (fully) be explained by a known organic pathology, these symptoms will be labelled 'medically unexplained' or 'functional'. Often, more than one symptom is present and certain constellations of symptoms give way to a diagnosis of a specific functional somatic (FS) syndrome like for example, chronic fatigue syndrome, fibromyalgia, or irritable bowel syndrome, with specific diagnostic criteria for each syndrome[1-3]. The Diagnostic and Statistical Manual of Mental Disorders version IV (DSM-IV-TR) offers classifications for severe and chronic heterogeneous FS symptom-presentation, such as somatoform disorder and somatisation disorder[4]. With a high prevalence of FS symptoms in primary and secondary care[5,6], research aimed at identifying predisposing and precipitating factors in the development of FS symptoms is highly relevant[7].

In this context, infections seem to play a role in the development of FS symptoms: Infections have been indicated as a precipitating factor in the onset of FS symptoms in a subset of patients[8-10], and a history of multiple infections (i.e. a high infection load) might predispose for the development of FS symptoms[11-13]. However, research on infections as a predisposing factor is still limited and results are inconsistent. Two studies reported multiple infections to be risk markers for having chronic fatigue syndrome or irritable bowel syndrome[11,12], while another study did not find chronic fatigue syndrome patients to have suffered from childhood illnesses more than controls[13]. These previous studies have some limitations. First, methodological choices may have influenced the results, such as the use of subjective reports of infections or illnesses[12,13], or dichotomizing the occurrence of infections (e.g. none vs. one or more infections)[11-13], thereby not taking into account the potentially accumulating effect of having had several infections. Another limitation of the above mentioned studies is the generalizability of their results, as they have focused mainly on patients with chronic fatigue syndrome and irritable bowel syndrome. Including a possibly more severe psychiatric patient group with somatoform disorder (based on the DSM-IV diagnostic criteria) may provide with more sensitivity for finding effects. Thus, more research on the role for infection as a predisposing factor of FS symptoms is warranted, including other FS symptoms patient groups such as somatoform disorder patients and encompassing a more elaborate and sensitive measure of infection load in the history of these patients (i.e. an objective, continuous measure of both major and minor infections).

In the current study, we estimated the infection load of patients with a DSM-IV somatoform disorder diagnosis, and we compared this load with the infection load of a control group in a matched case-control design. General practitioners' (GP) recordings of infections were collected prospectively up till the point of a somatoform disorder diagnosis. We created a continuous measure of infection load, including a broad range of infections. It was hypothesized that patients with a somatoform disorder would show more infections (i.e. a higher infection load) in their history than controls.

Having a somatoform disorder is a significant predictor of frequent GP attendance[14,15]. Theoretically, a high attendance frequency increases the chance for the GP to observe and record infections for which patients with a low attendance frequency would not visit their GP. Thus, one could argue that patients with a somatoform disorder might have more infection registrations in a GP registry than other GP attendees, without having actually experienced more infections. In addition to reporting the number of GP contacts per group (which is expected to be larger in the somatoform group), we aimed to control for this confounder by also creating a measure of infection load including only registrations for which anti-infectious or anti-inflammatory medication was prescribed, assuming that medication would only be prescribed for more severe infections (for which low attenders would also visit their GP). Secondly, as psychological distress predicts high GP attendance frequency[15,16], we also explored the influence of psychological distress (registrations of anxiety and/or depression) as a predictor of infection load.

Methods

For data collection, the Psychiatric Case Register Middle Netherlands (PCR-MN), a database covering all mental health care institutions in the mid-western part of the province of Utrecht, the Netherlands[17] was linked to the Julius General Practitioners Network (JGPN), a database encompassing over 300.000 residences in the Utrecht region for selection of the samples. The PCR-MN contains DSM classifications of patients who consult or are treated in mental health care institutions in the region. In all institutions that affiliated with the PCR-MN, patients are diagnosed by a trained psychologist or psychiatrist using standard tests (such as the SCID-I structured clinical assessment of DSM-IV axis I disorders)[17]. The JGPN contains GP registrations of medical diagnoses (coded according to the International Classification of Primary Case,

ICPC) and medication prescriptions (coded according to the Anatomical Therapeutic Chemical Classification System, ATC).

Samples

Two samples were selected: 1) persons who received a DSM-IV-TR diagnosis for an undifferentiated somatoform disorder, a somatization disorder, or a pain disorder and who were accordingly treated in a psychiatric setting (Somatoform Disorder, SD); 2) persons in the JGPN who were not in the SD group and who did not have a FS syndrome diagnosis or a record of FS complaints (Control).

SD cases were identified in the PCR-MN and were retained only if they could be identified in the JGPN as well. Controls were obtained from the JGPN although they could be present in the PCR-MN as well (see exclusion criteria below). Controls were matched to the available SD cases on gender, date of birth (± two years), postal code (to match for social economic status), and age in the period for which data in the JGPN was available (± five years).

General inclusion criteria were: at least three consecutive years of data available in the JGPN and no registered diagnosis of a schizophrenic disorder, psychotic disorder, or bipolar disorder in the PCR-MN. Inclusion criterion for the SD group was a DSM-IV-TR diagnosis (registered in the PCR-MN) between 2005 and 2010 of undifferentiated somatoform disorder, somatization disorder, or pain disorder. Criteria for exclusion in the Control group were a registration in the PCR-MN of undifferentiated somatoform disorder, somatization disorder, pain disorder, or conversion disorder. Further, controls were excluded in case of an indication for FS symptom presence in the JGPN: a registration of irritable bowel syndrome, chronic fatigue syndrome, fibromyalgia, or hysteria/ hypochondria, or at least three registrations within a time period of six months of either of the following: tiredness or weakness, general deterioration, muscle pain, or symptoms of several or not specified muscles, not followed by a diagnosis of a medical condition within 30 days after the registration. DSM-IV and ICPC-codes for in- and exclusion criteria are provided in appendix B.

Time periods over which registrations were obtained

Prospectively collected data in the JGPN were used to create the infection load measures. Relevant registrations within the JGPN were counted until the date of SD diagnosis or a matched date (± five years) for the Control group. For both the SD and

the Control group, registrations of infections were counted within the total time period before SD diagnosis for which data was available in the JGPN. Because this time period was expected to differ between cases, a year-average was computed. Infection load was also assessed for a time period that had the same length for all cases: the last three years before SD diagnosis for which data was available in the JGPN. This defines the variable 'three-year infection load'. The choice for three years was based on availability within the JGPN. Longer would have excluded too many cases, while a shorter period was expected to include not enough registrations to make comparisons. Note that the date of the SD diagnosis did not necessarily coincide with the end of the time periods; a gap could exist between the end of a time period and the date of SD diagnosis when a patient changed to a GP that was not included in the JGPN (see Figure 1). On average this gap was 11 months.

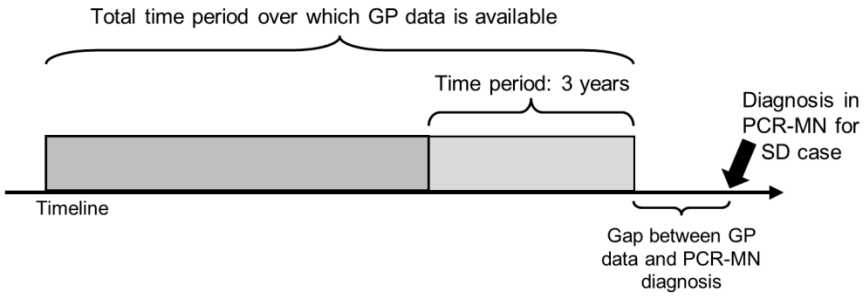

Figure 1. Schematic representation of the two time periods over which variables were obtained.

Infection load

The 'total infection load' was defined as the number of registrations of all infections within the JGPN. Further, four sub-categories of infection load were created: 1) localized infections commonly without fever and with recovery within days or weeks (mild infections; e.g., acute sinusitis, viral conjunctivitis), 2) localized infections commonly with fever and with recovery within days or weeks (severe infections; e.g., appendicitis, meningitis), 3) systemic infections with recovery within days or weeks (e.g., influenza, measles), and 4) chronic infections (e.g., chronic bronchitis, mononucleosis infectiosa).

A 'medication-treated infection load' was also created both for the 'total infection load' and for its sub-categories in both time periods. This load includes only infection

and inflammatory-related registrations that were accompanied by a relevant medication prescription (e.g. anti-viral, anti-bacterial, anti-parasitic or immune modulating). Thus, infection loads (total load and subcategories) were calculated within both time frames, including all infection registrations and including only medication-treated infection registrations.

Psychological distress

All registered diagnoses in the JGPN of anxiety disorder or state of anxiety, depression, reactive depression, and other/not specified depression were counted as a measure of psychological distress.

GP contacts

The number of days on which there was contact with the GP (e.g., by telephone, visit, etcetera) leading to a registration in the JGPN, was computed as a measure of total GP contacts.

Data analyses

All data analyses were performed with IBM SPSS statistics version 20. As all variables showed severe skewness which could not be improved by transformation, the Mann-Whitney U test for independent samples with Monte Carlo method for significance tests was used for group comparisons. Alpha (two-tailed) was set to .05 for comparisons of the total infection loads and to .01 (to account for multiple tests) for comparisons of the subcategories of infection load. For all significant tests, r ($z/\sqrt{N}$) is reported as an indicator of effect size.

The SD group was compared to the Control group on their infection load (total load and per subcategory; including all registrations and including only medication-treated registrations) in the total available time period (i.e. year-average infection load) and in the three-year time period (i.e. year-average infection load). Groups were also compared on the number of GP contacts and on psychological distress. The relation between psychological distress and infection load was analyzed within both groups to test whether psychological distress might account for group differences in infection load.

Results

Group characteristics

A flow-chart of the sample selection is shown in Figure 2. One hundred ninety-five SD cases could be identified in the JGPN of which 185 could be matched to a Control. Groups did not differ in age (mean age was about 44 years in both groups) or gender-ratio (67 % was female in both groups). The length of the total time period for which information was available in the JGPN varied considerably. On average, 10.5 years of data were available, with ranges of 3.2 – 45.9 for the SD group and 3.0 – 47.1 for the Control group. The groups did not differ in the length of the total time period (p = .27).

In the SD group, the time period between the last available date in the JGPN and date of the somatoform diagnosis (i.e. the 'gap' in Figure 2) showed considerable variation. On average, JGPN data was not available for a period of 11 months preceding the diagnosis with a range of 0 to 198.80 months (M [SD] = 10.95 [7.38] months),. Thirteen percent of the SD cases had a gap of five years or more and 4.9 % had a gap of ten years or more.

As expected, SD cases had more days with GP contacts than Controls in both time periods (total time period: mean ranks 227.56 vs. 143.44, U = 12614.0, z = -4.378, p < .001, r = .23; three-year time period: mean ranks 209.82 vs. 161.18, U = 13291.50, z = -5.567, p < .001, r = .29). On average, approximately 1 in every 25 GP contacts resulted in an infection registration in the SD group and 1 in 14 in the Control group, indicating that although the number of GP contacts was higher in the SD group, this seems not a probable cause for more infection registrations.

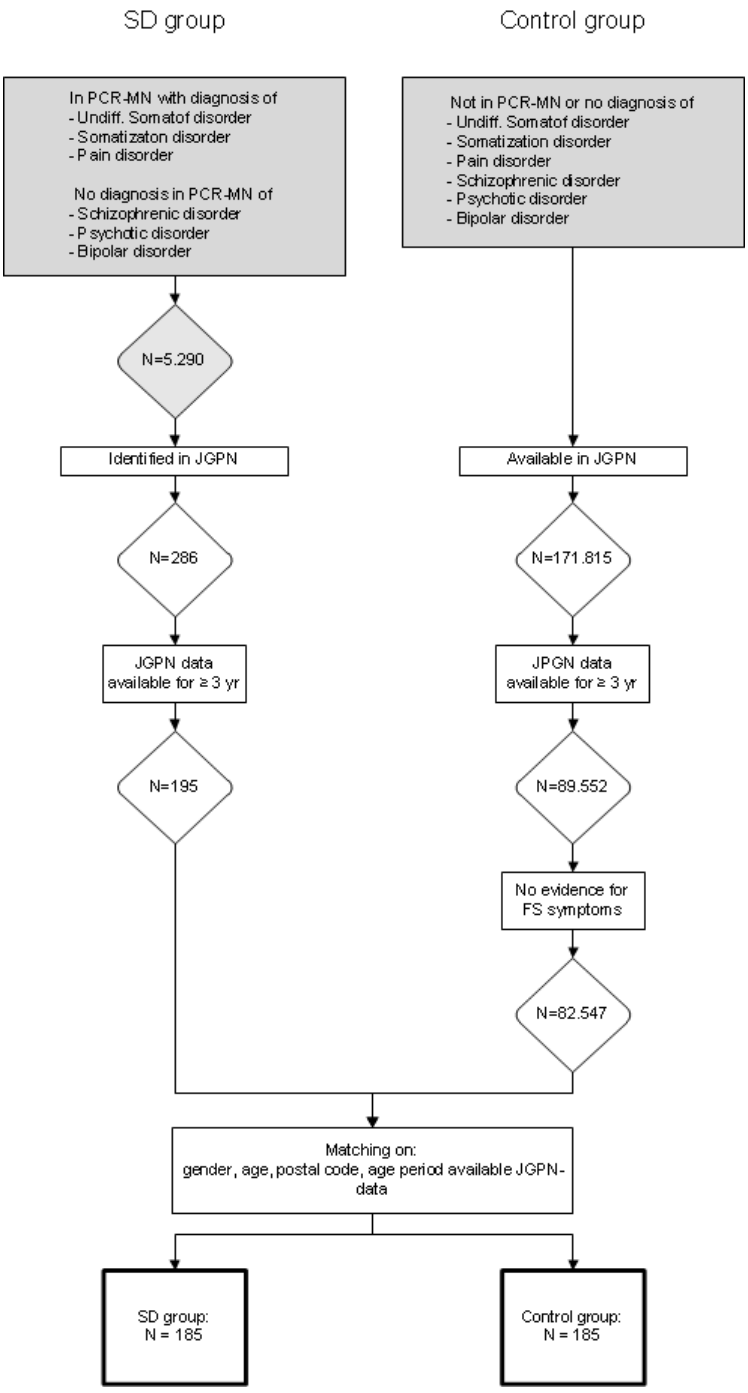

Figure 2. Flow-chart of sample selection in PCR-MN (top grey boxes) and JGPN (white boxes).

Year-average infection load

The upper half of Table 1 shows the year-average total and medication-treated infection load and subcategories of infection loads.

Total infection load

The year-average total infection load and medication-treated infection load were significantly higher in the SD group compared to the Control group (total infection load: mean ranks 202.55 vs. 168.45, r = .16; medication-treated infection load: mean ranks 197.23 vs. 173.77, r =.11).

Infection load per category

SD cases had a significantly higher year-average of severe infections than Controls (year-average: mean ranks 201.81 vs. 169.19, r = .16; year-average medication-treated: mean ranks 198.08 vs. 172.92, r = .14). Groups did not significantly differ on the other subcategories of infection load within the total time period.

Three-year infection load

The lower half of Table 1 shows the three-year total and medication-treated infection load and sub-categories of infection loads.

Total infection load

The three-year total infection load and medication-treated infection load were significantly higher in the SD group compared to the Control group (total infection load: mean ranks 200.89 vs. 170.11, r = .15; medication-treated infection load: mean ranks 195.66 vs. 175.34, r = .11).

Infection load per category

No group differences were found for any of the subcategories of infection load or medication-treated infection load within the three-year time period.

Table 1. Descriptives (M (se), range) and test results for the inflammatory load in the total available time period (averaged over number of available years) and in the three-year time period.

		SD group (n = 185)	Control group (n = 185)	Mann-Whitney U test
Year-average	Total inflammatory load	0.87 (.10), 0 - 11.73	0.51 (.06), 0 - 7.97	U = 13958.5, z = -3.08, p = .002
	Mild infections	0.52 (.07), 0 - 6.17	0.28 (.04), 0 - 5.89	U = 14604.5, z = -2.48, p = .012
	Severe infections	0.29 (.04), 0 - 5.25	0.18 (.02), 0 - 2.01	U = 14096.0, z = -3.08, p = .002
	Systemic infections	0.03 (.01), 0 - 0.86	0.04 (.01), 0 - 1.24	U = 16457.0, z = -1.10, p = .27
	Chronic infections	0.04 (.01), 0 - 2.35	0.01 (.00), 0 - 0.29	U = 16160.5, z = -1.76, p = .068
Year-average medication treated	Total inflammatory load	0.36 (.05), 0 - 4.01	0.21 (.03), 0 - 3.43	U = 14942.0, z = -2.18, p = .027
	Mild infections	0.24 (.03), 0 - 2.96	0.14 (.02), 0 - 2.68	U = 15648.0, z = -1.53, p = .13
	Severe infections	0.10 (.01), 0 - 1.23	0.06 (.01), 0 - 0.75	U = 14786.0, z = -2.77, p = .007
	Systemic infections	0.01 (.00), 0 - 0.21	0.01 (.00), 0 - 0.51	U = 17010.0, z = -0.28, p = .69
	Chronic infections	0.02 (.01), 0 - 1.18	0.00 (.00), 0 - 0.13	U = 16351.0, z = -1.99, p = .026
Three-year time period	Total inflammatory load	3.44 (.47), 0 - 42	2.15 (.50), 0 - 84	U = 14265.5, z = -2.91, p = .004
	Mild infections	2.09 (.36), 0 - 42	1.35 (.37), 0 - 62	U = 15033.0, z = -2.26, p = .025
	Severe infections	1.13 (.19), 0 - 18	0.70 (.16), 0 - 22	U = 15049.0, z = -2.51, p = .013
	Systemic infections	0.08 (.03), 0 - 2	0.06 (.03), 0 - 5	U = 16744.5, z = -1.02, p = .35
	Chronic infections	0.15 (.06), 0 - 9	0.03 (.01), 0 - 1	U = 16267.5, z = -2.15, p = .023
Three-year time period medication treated	Total inflammatory load	1.53 (.24), 0 - 25	0.99 (.20), 0 - 30	U = 15233.0, z = -2.09, p = .040
	Mild infections	1.03 (.19), 0 - 23	0.72 (.16), 0 - 23	U = 15977.5, z = -1.35, p = .18
	Severe infections	0.43 (.09), 0 - 10	0.24 (.06), 0 - 7	U = 15856.5, z = -1.92, p = .052
	Systemic infections	0.01 (.01), 0 - 1	0.02 (.01), 0 - 1	U = 16927.5, z = -1.01, p = .63
	Chronic infections	0.07 (.03), 0 - 4	0.01 (.01), 0 - 1	U = 16463.0, z = -2.15, p = .045

Effects of timing on infection load

To get an impression of the influence of the length of the gap between available GP data within the JGPN and the psychiatric diagnosis in the PCR-MN, SD cases with a gap ≥ 5 years (n = 23) and SD cases with a gap < 5 years (n = 162) were compared on the total infection load measures. Mann-Whitney U tests showed that SD cases whose infection load was measured more closely to the date of the somatoform diagnosis had a higher infection load (all *p*-values < .05).

Psychological distress

The number of anxiety and depression-related registrations in the JGPN was higher in the SD group than in the Control group in both time periods (total time period: mean ranks 210.17 vs. 160.83, $U = 12548.5$, $z = -6.622$, $p < .001$, $r = .34$; three-year time period: mean ranks 206.15 vs. 164.85, $U = 13291.50$, $z = -5.567$, $p < .001$, $r = .29$).

Within the Control group, all associations between the number of anxiety and depression-related registrations and the total and medication-treated infection load measures were significant (year-average total infection load: $\rho = .21$, $p = .005$; year-average medication-treated infection load: $\rho = .25$, $p = .001$; three-year infection load: $\rho = .20$, $p = .006$; three-year medication-treated infection load: $\rho = .25$, $p = .001$). Within the SD group however, associations were found only for the infection loads not restricted to medication-treated infections (year-average total infection load: $\rho = .18$, $p = .014$; three-year total infection load: $\rho = .18$, $p = .014$).

Discussion

We aimed to address the question whether a high infection load could be a predisposing factor in the development of a somatoform disorder. We found that patients with a somatoform disorder indeed had a higher infection load in the time period preceding the psychiatric diagnosis. This was true both in the total time period for which data was available and in a three year time period that was as close to the diagnosis as possible. Our and other's[11,12] findings point to a role for the immune system in the development of FS symptoms and somatoform disorder. This is in line with the immunological perspective on FS symptoms suggesting a role for immune-brain communication[18]. On activation of the innate immune system, immune-brain communication by way of cytokines acting on the brain results in several changes in

behavior and mood, together called sickness behavior[19-21]. Animal research shows that repeated immunological (or psychological) stressors can sensitize immune-brain communication, resulting in a more pronounced expression of sickness behavior in response to new (mild) stressors[22], even in absence of increased peripheral immunological activity[23]. Theoretically, it is possible that this sensitization of immune-brain communication due to multiple infections occurs in humans as well, leading to a chronic expression of sickness behavior i.e., FS symptoms. We have shown here that patients with somatoform disorders indeed have a higher infection load than matched controls in the time preceding their diagnosis, indicating the relevance of further research addressing immune-brain communication pathways in these and other FS patients.

Between-group comparisons of subcategories of infection load indicated that differences were mainly found in the number of acute, localized infections that are commonly accompanied by fever. As cytokines acting on the brain are also responsible for the occurrence of fever[24], fever during an infection would implicate immune-brain communication and thus the expression of sickness behavior. The occurrence of minor infections might not add to the infection load in SD patients because the absence of (cytokine-induced) fever during these infections indicates a more local immunological response, possibly not affecting immune-brain pathways so much.

Patients with available GP data relatively close to the date of SD diagnosis (i.e. a small time gap) had a higher infection load than patients with available GP data more distant from the SD diagnosis. Thus specifically more recent infections elevate the chance for developing somatoform symptoms. This is in line with Hamilton[11], who found that recent infections (here defined as one year before diagnosis) posed a higher risk factor for developing a fatigue syndrome than more distant infections (1 - 3 years before diagnosis).

Beforehand, we considered the possibility that between-group differences in infection load could be an artefact of differences in GP attendance. As expected, we indeed found that SD patients had more GP contact-days than matched controls, although the question remains whether the number of GP contacts has influenced the number of infection registrations or that having more infections was the cause of more frequent GP visits. The findings in our study that SD patients also had a higher infection load when compared on the more stringent medication-treated infection load, and that psychological distress was not related to the medication-treated infection load in the SD

patient group, support the conclusion that the group differences found in infection load are a genuine effect. No convincing evidence was found for GP attendance to underlie a higher infection load in SD patients.

Some limitations of the study need mentioning. First, despite having access to large registry databases for creating our two groups, the final samples were not as large as hoped for. Together with the need for non-parametric tests, which have less power than their parametric equivalents, it is possible that we lacked power to detect smaller differences. This is a limitation specifically for interpretation of probable more subtle group differences in the subcategories of infection load. Second, our study solely included somatoform disorder patients who received their diagnosis at a mental health care institution. This is a specific sample of patients who decided to seek (or accept) psychological or psychiatric help for their symptoms, possibly indicating that this sample experienced more severe and/or heterogeneous FS symptoms and higher levels of psychological distress compared to patients who did not seek psychiatric help. It may be argued that including a somatoform disorder patient group results in larger effects for the expected group differences, which is especially an advantage since our sample was smaller than expected. A disadvantage is that we cannot generalize our findings to other FS symptom patient groups. Therefore, a meaningful next step would be a replication of our results in other FS symptom patient samples. Last, as only GP registrations were used, it is possible that some specific infections were not accounted for in our measure of infection load. For example, oral infections are more commonly detected by a dentist and they are thus underrepresented in our measure of infection load.

In conclusion, we found a higher number of infection registrations in patients with somatoform disorder compared to matched controls, mainly driven by differences in severe acute local infections. We found no evidence for GP-attendance to underlie these differences. It is therefore concluded that patients with a somatoform disorder indeed have a higher infection load preceding their diagnosis. These findings point to a role for immune-brain communication in FS symptom development.

Acknowledgments

We gratefully acknowledge Janneke Giele, PCR-MN data-manager, for her assistance with the data.

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Chapter 7.

Negative affectivity predicts increased pain sensitivity during low-grade inflammation in healthy women

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Abstract

Introduction: Sensitization of immune-to-brain pathways has been proposed as a mechanism underlying medically unexplained (i.e., functional) somatic symptoms. Aim of the present study was to assess the interaction between stress (life events and perceived stress) or stress vulnerability (negative affectivity) and an immunological challenge on experimentally determined pressure pain sensitivity (threshold and tolerance). ***Methods:*** Healthy female participants (*n* = 25, mean age 22.3 years) reported life events, perceived stress and negative affectivity by questionnaire and all participants received a *Salmonella typhi* vaccine and saline control in a randomized cross-over design. Pressure pain threshold and tolerance were assessed before and six hours after each injection. ***Results:*** Vaccination induced leukocytosis and increased IL-6. Negative affectivity was significantly related to decreased pain tolerance after vaccination, but not after placebo.

Discussion: Negative affectivity predicts enhanced pain sensitivity during low-grade inflammation, suggesting that this vulnerability factor may reduce the threshold for immune-to-brain communication in humans.

Introduction

In reaction to an immunological challenge, immune-to-brain communication leads to a constellation of reversible behavioral and affective changes, denoted as sickness behavior[1,2], which is considered an adaptive response supporting immunity and recuperation[3]. Characteristic features are decreases in food intake and explorative behavior, impaired learning, anhedonia, and increased pain sensitivity[4,5]. Results from animal models now point to the possibility of sensitization of immune-to-brain communication by repeated exposure to immunological challenges or a combination of psychosocial and immunological challenges. Psychosocial factors may sensitize immune-to-brain communication leading to a prolonged or more severe expression of sickness behavior symptoms[6-9], pointing to cross-sensitization of the systems. For example, there is evidence that increased pain sensitivity after an immunological challenge is expressed more strongly on the backdrop of a prior stressor[10]. Furthermore, such sensitization may be compounded by individual characteristics related to stress coping; this was nicely illustrated by a study of Gibb et al.[11], in which the synergistic effects of a social stressor and an immunological stressor on sickness behavior symptoms were more pronounced in mice from a relatively high stress reactive strain as compared to a low stress reactive strain.

The sensitization of immune-to-brain pathways might be applicable to humans as a possible explanatory mechanism for somatic symptoms that cannot be fully explained by a present medical condition (i.e., functional somatic symptoms)[12]. Indeed, many common functional somatic symptoms in humans, such as pain and fatigue, resemble symptoms of sickness behavior observed in animal studies. This idea is consistent with the observation that onset of functional symptoms is often related to an immunological stressor[13,14]. Furthermore, a pattern of multiple psychosocial stressors in the past has frequently been linked to functional somatic symptoms; chronic unexplained fatigue (i.e. chronic fatigue syndrome) has been linked with childhood trauma[15], and chronic unexplained widespread pain (i.e. fibromyalgia) with serious life events[16]. Finally, increased stress vulnerability has been linked to symptom experience in general. Especially the trait negative affectivity (NA) has been related to symptom report[17-19]. High levels of NA are related to a tendency to negatively interpret information and to higher emotional reactivity to negative events[20]. Sensitization of immune-to-brain communication can theoretically explain the observed relation between stress or stress vulnerability and symptom experience. Therefore, obtaining insight into the

mechanisms leading to sensitization of immune-to-brain pathways in humans is a necessary next step.

Although previous studies have tested the effects of an immunological challenge on sickness behavior in humans[21-23], only few studies have focused on the synergistic effects of psychosocial and immunological stressors. Brydon et al.[24], showed that the combination of an acute experimental psychosocial stressor and vaccine administration leads to a stronger immunological response and increase in negative mood than vaccine exposure alone. Strike et al.[25] reported financial strain to be related to changes in negative mood during vaccine-induced inflammation. However, expressions of other sickness behavior symptoms such as pain sensitivity were not assessed in these studies. Thus, human studies provide preliminary confirmation of findings from animal studies showing synergistic effects of psychosocial and immunological stressors, which warrant further research. In going with evidence from animal studies, such research should also consider stress sensitivity traits, which thus far have not been taken into account.

In light of the preceding discussion, the goal of the current study was to further investigate the possible synergistic effects of psychological stress or stress vulnerability and immunological stress on pain sensitivity in humans. Contrary to other sickness behavior symptoms like mood and fatigue (aspects which are typically measured through self-report), pain sensitivity can be assessed in a more objective manner by applying noxious stimuli (see also a study by Benson et al.[26]).

Our aim was to assess the interaction between relatively long-term stress (life events in the past year; perceived stress in the past two years) or stress sensitivity (NA) and a *Salmonella typhi* vaccination on markers of inflammation and pain sensitivity. We hypothesized that the occurrence of life events and/or high stress sensitivity would enhance the effects of the immunological challenge on pain sensitivity. The present study was limited to women since both psychosocial distress and functional somatic symptoms are more common among them.

Methods

Participants

Healthy non-smoking female participants were recruited from among students and staff of the University of Birmingham by poster advertisement and word of mouth. Inclusion criteria were an age between 18 and 50 years old and use of oral contraception. Excluded were volunteers reporting diabetes mellitus, asthma, congestive heart failure, any psychiatric disorder, recent history of cancer, inflammatory disease, cardiovascular disease, chronic obstructive pulmonary disorder, wearing a pacemaker, and those taking any medications with immune modulatory responses. Finally, participants were excluded if they were unwell on the day of testing or fell ill between the two test-days. Eligible persons received either a monetary incentive or credits for participation.

Study design

Salmonella typhi capsular polysaccharide vaccine (0.025 mg in 0.5 ml, Typhim Vi, Sanofi Pasteur, UK) was used as the immunological challenge. In a placebo-controlled cross-over design, participants were tested in two conditions (i.e. vaccination and saline (placebo)) on two test-days with at least seven days in-between. The order of conditions was counterbalanced across the participants. Participants were blinded to the conditions. Dependent measures were taken on each test-day before injection and six hours post-injection. Stress and stress sensitivity measures were obtained during a separate appointment.

Protocol

Informed consent was obtained on an initial appointment, followed by screening for inclusion and exclusion criteria and assessment of perceived stress, life events, and NA with questionnaires (see below). Testing-days were scheduled based on the schedule for oral contraceptive use, thereby reducing variance in anti-conceptive hormones within and across participants. More specifically, participants with monophasic pills were tested during the three weeks of pill use and participants with either biphasic or triphasic pills were tested within the first ten days after a stop week. With the exception of the injection (vaccine or saline), both test-day protocols were identical. Participants reported between 8.00 and 11.00 a.m. at the Behavioral Immunology Laboratory of University of Birmingham, upon which baseline measures were obtained of heart rate,

blood pressure, body temperature, and pain sensitivity. A blood sample was obtained approximately 15 minutes after arrival. Next, the participant performed on cognitive tasks (data not reported here). Following these assessments, the participant was injected with either vaccine or saline and rested for 30 minutes. All morning measurements were repeated six hours post-injection, which coincides with a peak increase in inflammatory markers[27]. Participants were allowed to leave the laboratory in between test-sessions with instructions regarding diet. The protocol was approved by the Health Research Authority NRES Committee West Midlands – South Birmingham.

Physiological measures

Blood pressure and pulse rate were measured using an electronic sphygmomanometer. Body temperature was measured with a digital ear thermometer. Blood samples were obtained by venipuncture in the participant's arm and spun down immediately after testing. Analyses for full white blood cell (WBC) count were done using a Coulter Analyzer (Beckman Coulter, Inc.). Serum was analysed for the inflammatory markers interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) using high sensitivity ELISAs (Quantikine HS Human IL-6 ELISA and Quantikine HS Human TNF-α, both R&D Systems, UK) in accordance with the manufacturer's instructions. The reported sensitivity of the assays was 0.039 pg/ml and 0.106 pg/ml for IL-6 and TNF- α respectively, with recorded intra-assay and inter-assay variations both <10%.

Psychosocial stress

Perceived stress (past two years) was assessed with the Perceived Stress Questionnaire (PSQ), a 30-item questionnaire on feelings of distress. Psychometric properties of the scale are good and the general PSQ shows moderate correlates with mood questionnaires and other stress scales[28].

Life events (past year) were measured with the Life Events Scale for Students (LESS)[29]. The occurrence of 36 student-typical life events in the last year had to be indicated on a dichotomous scale ("yes" (1)/"no" (0)). Events that were reported to have occurred were multiplied by the mean weighted rating for British samples[30], thereby giving the events a mean weight relative to the event "death of a parent". A sum score was then computed for all weighted ratings of reportedly occurred events.

Negative affectivity

NA was measured with the NA subscale of the Positive and Negative Affect Scale (PANAS)[31]. The NA subscale includes 10 feelings and emotions for which the extent to which the emotion was felt in the past month had to be rated. Psychometric properties of the subscale are good (α for 'Past few weeks' instructions = .87)[31].

Pain sensitivity

Pressure pain threshold and pressure pain tolerance were measured by algometry using a digital pressure algometer (FPX50; Wagner Pain Test™, Greenwich, USA). For the pressure pain threshold, gradually increasing pressure was applied at a rate of 100 kPa/s until the participant indicated the force to become unpleasant, upon which the pressure was immediately released. Pain thresholds were assessed three times at four body points: bilaterally at the calf muscle belly and on the lower back at the paraspinal muscles of L3. A mean pain threshold was calculated by averaging over the last two measurements of all body points.

Pressure pain tolerance was measured on the thumbnail of the non-dominant hand by gradually increasing pressure at a rate of 100 kPa/s until the participant indicated the force to become unbearable. To apply this stimulus in a controlled manner, thereby standardizing this test across time intervals and participants, the algometer was placed in a wooden apparatus as described elsewhere[32]. Pain tolerance measurements were repeated (with a minimum of 30 s in between measurements) until the participant indicated that the pain tolerance level was reached. The highest obtained pressure was used as pain tolerance in the analyses.

Algometric pain sensitivity measures show good test-retest reliability for tests repeated within one session (intraclass correlation coefficient (ICC) > .80)[32,33] and over days (ICC > .70)[33,34].

Statistical analyses

The questionnaires did not have any missing data (with the exception of the LESS item parent losing job", *n* = 6)' and normality was adequate for all scales without visual outliers. IL-6 and TNF-α plasma concentrations were log transformed to gain a normal distribution, after which no severe outliers were detected. Pain threshold measures showed moderate skewness and were square root transformed. Transformed and non-transformed data yielded similar results and the latter were reported here.

The effects of vaccination were analysed with a 2 Condition (saline, vaccination) x 2 Time (pre-injection, post-injection) repeated measures analyses of variance (rm-anova), followed by post hoc analyses using paired samples t-tests. Alpha was set at .05.

The effects of perceived stress, life events, and NA were analysed by adding these factors as covariates to the model, resulting in 2 x 2 rm-ancovas. Separate analyses were performed per covariate. In case of significant three-way interactions (Condition x Time x Stress), response-variables were created for the dependent variable of interest by subtracting pre-injection scores from post-injection scores resulting in Δ-saline and Δ-vaccine change-score variables. Separate regression analyses were then performed with the Δ-variables as dependent variable and the stress factor of interest as predictor. To account for multiple testing, alpha was set to .016 for the rm-ancovas and to .025 for the post hoc regression analyses.

After testing of the first 18 participants, the vaccine batch that was used was recalled by the manufacturer because of potential low antigen content and vaccines from a different batch were used for the remaining nine participants. Therefore, batch was included as additional factor in all analyses. Data were analysed using SPSS for windows 20.1 (IBM SPSS Inc, Chicago).

Results

Sample characteristics

Twenty-seven participants were included in the study. Two participants were excluded from the analyses because of illness onset in between test-days. Mean age of the remaining sample (n = 25) was 22.28 years (SD: 3.39, range: 18-30). Mean BMI was 22.90 (SD: 2.76, range: 18.93 - 29.74), 6 participants (24 %) were overweight with a BMI > 25.

Five participants reported back pain on at least one of the testing days. Analyses on mean pain thresholds for the calf and lower back separately (not shown) did not differ from analyses on the pain threshold averaged over all anatomical sites. The two pain sensitivity measures correlated low to moderate (highest r = .60, p = .002). Table 1 shows descriptives for perceived stress, life events, and NA.

Table 1. Mean (SE) scores and ranges for the stress variables and NA (n=25).

	Mean (SE)	Range
Perceived stress (PSQ)	71.80 (2.56)	52 – 94
Life events (LESS)	283.56 (25.12)	73 – 495
NA (PANAS)	19.40 (1.19)	10 – 33

Effects of vaccination on autonomic measures and inflammation

Vaccination did not affect blood pressure (BP), heart rate, or body temperature (condition-by-time interactions on systolic BP: F (1,23) = 1.68, p = .21; diastolic BP: F (1,23) = 2.65, p = .12; heart rate: F (1,23) = 0.15, p = .70; body temperature: F (1,23) = 0.42, p = .53), which is in line with earlier reports on the effects of *Salmonella typhi*.

RM-ANOVA revealed a significant condition-by-time interaction for WBC (F (1,23) = 42.19, p < .001) and logIL-6 (F (1,23) = 48.81, p < .001). Post-hoc analyses confirmed that WBC increased in both conditions (p's < .001), but more in the vaccine condition (mean ±SE,; from 5.80 ±0.27 to 10.57 ±0.67 10^9/L as compared to saline (from 5.82 ±0.36 to 7.21 ±0.39 10^9/L). IL-6 significantly increased in the vaccine condition (untransformed mean ±SE: from 0.84 ±0.25 to 5.66 ±0.67 pg/ml; p < .001), but not in the saline condition (from 1.77 ±0.57 to 1.69 ±0.47 pg/ml; p = .96). No significant condition-by-time interaction was found for TNF-α (vaccine: from 3.89 ±1.23 to 6.45 ±2.00 pg/ml, saline: from 2.00 ±0.67 to 5.37 ±1.87 pg/ml; F (1,23) = 0.88, p = .36).

Vaccine-by-stress interactions on inflammatory markers

Perceived stress, life events, and NA did not interact with condition and time in the models with LogIL-6 or LogTNF-α as dependent variables (p's > .016).

Effects of vaccination on pain sensitivity

Figure 1 shows the change in the pain sensitivity measures (threshold and tolerance) within both conditions. No main effects (p's > .05) and no condition-by-time interaction effect (F (1,23) = 1.17, p = .29) were found for changes in pain threshold. A main effect of time was found for pain tolerance (F (1,23) = 4.92, p = .037), but no condition-by-time interaction (F (1,23) = .06, p = .80).

Vaccine-by-stress interaction effects on pain sensitivity

NA significantly interacted with condition and time on pain tolerance (F (1,22) = 9.45, p = .006). Separate regression analyses on the relation between NA and Δ-pain tolerance within both conditions showed a significant relation in the vaccine condition (β = -.57, t (22) = -2.96, p = .007) but not in the saline condition (β = .25, t (22) = 1.16, p = .26) (Figure 2). Thus, increased NA was related to a decrease in pain tolerance (i.e., a negative Δ-score) between pre- and post-injection in the vaccine condition. In other words, participants high on negative affect exhibited a lowered pain tolerance after vaccination than those low on negative affect.

Perceived stress and life events did not interact with condition and time on pain tolerance and none of the stress factors (NA, perceived stress and life events) showed an interaction with condition and time on pain threshold (p's > .016).

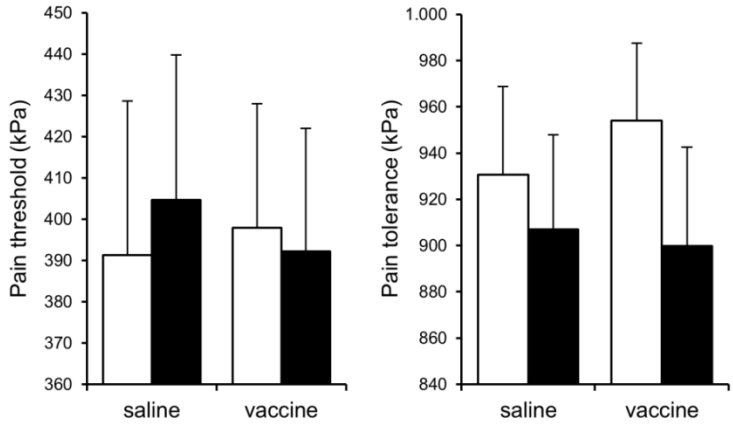

Figure 1. Within condition changes in pain threshold (left) and pain tolerance (middle) (white bars = pre-injection; black bars = post-injection). Depicted are means with SE.

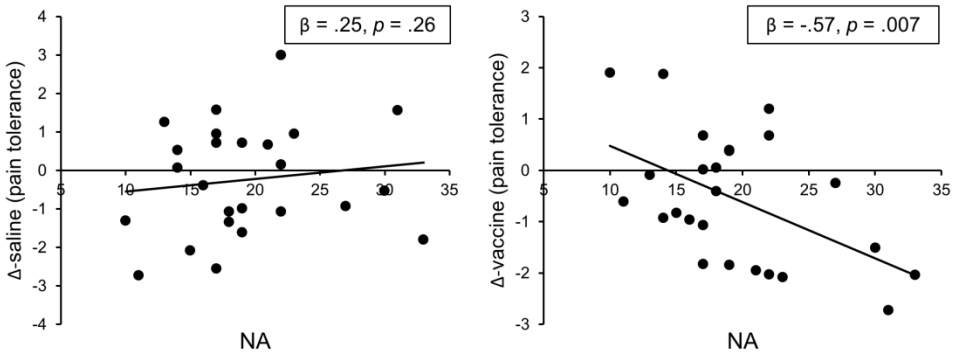

Figure 2. Scatterplot depicting the relation between NA (x-axis) and the change in pain tolerance (post-injection – pre-injection) within the saline condition (left panel) and the vaccine condition (right panel).

Discussion

The aim of this study was to assess interaction-effects between psychosocial stress and/or stress-vulnerability and low-grade inflammation on pain sensitivity in healthy human female participants. By experimentally inducing low-grade inflammation in healthy persons, it was possible to reduce random between-participant variance, thereby allowing for more reliable results. The immunological challenge (*Salmonella typhi* vaccine) induced the expected low-grade inflammation but did not show an overall effect on pain sensitivity as measured with algometry. NA was found to be a predictor of vaccine-induced changes in pain tolerance. In other words, low-grade inflammation appears to increase pain sensitivity specifically in persons with high NA.

NA is a measure of subjective distress and unpleasurable engagement[31] and is strongly related to neuroticism[35]. Neuroticism is related to daily-life perceived stress[36] and considering the strong relation between NA and neuroticism, NA is most likely also related to perceived stress. Our finding, however, that NA, but not the amount of stressors (such as life events) predicts vaccine-induced changes in pain sensitivity suggests that it is especially the negative-affective interpretation of potential stressful events that sensitizes for subsequent sickness behavior responses to (low-grade) inflammation. Close inspection of the PSQ shows that items are a broad mixture of somatic and psychosocial experiences[28]; this might possibly explain why no effect was found for the PSQ. More research, however, is needed to obtain further insight in the specificity of NA.

The effects of systemic inflammation on pain sensitivity have been well described in animal models with as key mechanism the activation of glia cells by pro-inflammatory markers, which in turn alter neuronal functioning along the pain pathway[37]. Furthermore, inflammation-induced pain has repeatedly been shown to interact with exposure to multiple stressors in rodents[10,38]. To our knowledge, our results are the first evidence from human studies that NA and inflammatory factors can also interact to induce increased pain sensitivity, reflecting that NA may reduce the threshold for sickness behavior in humans. Since we did not find any effects of NA on vaccine-induced changes in peripheral inflammation, NA probably interacts with central aspects of immune regulation. A recently suggested mechanism that might explain how NA (and self-perceived stress) acts on central immune regulation describes how stress-related increases of glucocorticoids (which normally have an anti-inflammatory effect) can also enhance inflammatory responses to new stressors, probably by sensitization

of microglia[39]. Microglia are key modulators of immune-brain communication and also play a role in central processing of peripheral pain[40]. High NA individuals might (as a result of frequent self-perceived stress) have primed microglia cells for new challenges. Thus, while a mild immunological challenge (i.e. vaccination) applied in the current study did not lead to an inflammatory response potent enough to induce increased pain sensitivity in all participants, the low-grade inflammation was enough to induce a central inflammatory response in participants in which the central immune response might have been already sensitized, i.e. in participants with high NA.

In our interpretation, our results are suggested by a model in which inflammation increases pain sensitivity specifically in high NA persons. The results, however, might also fit a model in which inflammation facilitates the (well-known) relationship between NA and symptom experience. The observation that not all individuals with NA experience somatic symptoms indicates that an additional factor is needed, which may very well be the occurrence of an infection.

Interestingly, NA predicted vaccine-induced changes in pain tolerance but not pain threshold. Although pain threshold and pain tolerance can both be considered measures of pain sensitivity, there is evidence that they represent different dimensions of the construct. An earlier study using the same methodology for measuring pain threshold and pain tolerance showed that pain tolerance is only slightly related to pain threshold but moderately related to subjective sensory and affective pain ratings[32]. Pain threshold on the other hand showed low correlations with sensory and affective pain ratings. Thus, pain tolerance seems to incorporate more of an affective (or emotional) aspect of pain sensitivity, while pain threshold seems to represent a (more objective) sensory aspect. This distinction between the pain sensitivity measures is also illustrated by the low to moderate correlations found between the measures in the current study. Thus, the interaction effects of NA and inflammation seems to be specific for the emotional aspects of pain sensitivity. It has been suggested that sickness behavior is a motivational state, a “central state that reorganizes perception and action”[41]. In this view, it makes sense that mainly the affective or emotional aspects of pain sensitivity change during inflammation, as it are these aspects that influence the individual’s withdrawing behavior needed to recuperate from the infection.

Limitations and recommendations

Two different batches of the *Salmonella typhi* vaccine were used, thereby enhancing between-participants variance in immunological activity. However, batch was included as a factor in all analyses and showed no effect in most analyses. The sample used in this study was relatively small, leading to reduced power to detect smaller effects. It is possible that in a larger sample, additional stress(-vulnerability) factors will be found to be significant predictors of vaccine-induced changes in pain sensitivity. As only healthy females participants were included, this reduces generalizability of our results. For example, gender differences have been reported for pain sensitivity[42] and it is possible that gender differences also exist in inflammation-induced changes in pain sensitivity.

Conclusion

In summary, NA, a trait related to the negative or stressful interpretation of events, was found to affect vaccine-induced changes in pain sensitivity in a healthy female sample. As NA was not related to the inflammatory markers, it was concluded that NA may enable priming of microglia cells that serve as intermediates between the peripheral and the central immune system, thereby reducing the threshold for immune-to-brain communication.

Acknowledgements

We thank Lotte Lintmeijer and Nicola Paine for their excellent work during data acquisition.

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Chapter 8.

Summary & Prospects

Summary

Part I of this thesis addressed some methodological issues regarding research on immune-to-brain communication in FS symptom patients. The first issue was whether specific subgroups of FS symptom patients really exist. Patients with FS symptoms are often diagnosed based on specific symptom patterns and these diagnoses are generally also used to include FS symptom patients in research. The validity of differentiating between patients based on these symptom patterns (i.e. the splitters' perspective) is, however, a matter of debate. According to the study results presented in **Chapter 2**, differences between FS symptom patients are mostly seen in overall symptom severity: the mean symptom score on a heterogeneous list of symptoms. These results support the so called lumpers' perspective, with the implication that patients should be included in research based on criteria of overall symptom severity. On the other hand evidence was also found for some specificity of symptom patterns in a subgroup of patients with moderate levels of fatigue or pain. This implies that studies can also be focussed on specific subgroups of patients. Nevertheless, although the traditional approach of studying symptom-specific subgroups may have some value, our results mostly support the inclusion of a sample with heterogeneous symptoms in future research.

The second issue in part I was the methodology for pain sensitivity assessment. Algometry is often used to assess pressure pain sensitivity in patients with chronic pain complaints, but standardized methodology is lacking. We provided a protocol for assessing several aspects of pain sensitivity through algometry in **Chapter 3**. Alongside procedural recommendations, it was also shown in this chapter that pain threshold and pain tolerance should be regarded as different aspects of pain sensitivity and that both measures should therefore be included in studies on pain sensitivity. This notion was emphasized by the findings in **Chapter 7**, where indeed different results were found for pain threshold and pain tolerance.

The third issue in part I was the need for additional (objective) measures of fatigue. Research on fatigue is generally restricted to questionnaires, especially in the assessment of cognitive fatigue which is seen as an aspect of central fatigue (i.e., contrary to physical fatigue which also has a peripheral aspect). Objective measures, however, are needed to obtain a reliable and valid assessment of the

cognitive aspect of fatigue. Despite promising results from earlier studies on changes in cognitive performance and mental effort allocation (as assessed with fMRI) during cognitive fatigue, we could not find evidence for a reflection of fatigue in cognitive performance and mental effort allocation as assessed with pupillometry in two separate studies (**Chapter 4**). However, methodological limitations in these studies imply that further research is needed for a conclusive answer.

Part II of this thesis presented preliminary evidence for sensitized immune-to-brain communication underlying FS symptoms. First, stress-related factors (state anxiety as a psychological manifestation of stress and cortisol as a stress hormone) and gastrointestinal symptoms suggestive of low-grade inflammatory processes were found to predict experimentally determined visceral pain sensitivity in healthy women (**Chapter 5**). Thus, pain sensitivity appears to be affected both by psychological stress-mechanisms and by immunological mechanisms, suggesting the possibility of synergistic effects of psychological and immunological factors in increasing (sensitized) pain sensitivity.

Second, the results presented in **Chapter 6** showed that patients with a somatoform disorder diagnosis (presenting with severe and chronic FS symptoms) had experienced more infections in the past than had a matched control group without FS symptoms. These findings support the hypothesis that patients with a somatoform disorder may have developed a sensitized immune-to-brain communication as a result of frequent exposure to immunological stressors.

Third, a preliminary experimental demonstration of the synergistic effect of stress vulnerability and an immunological stressor on subsequent sickness behavior in healthy subjects was presented in **Chapter 7**. Specifically, it was shown that negative affectivity, related to a tendency to negatively interpret information and to higher emotional reactivity to negative events[1] is related to increased pain sensitivity after administration of a vaccination. This shows that sensitization of immune-to-brain communication as well as subject-specific vulnerability may also be present in humans.

In sum, preliminary evidence is provided here supporting the theory of sensitized immune-to-brain communication underlying FS symptoms. It was shown that infection load predisposes for the experience of severe chronic FS symptoms

in patients with a somatoform disorder, that both anxiety (a psychological stress response) and cortisol (a stress hormone) and symptoms suggestive of low-grade inflammation are related to an important sickness behavior measure (i.e. pain sensitivity) in healthy subjects, and that negative affectivity (stress-vulnerability) interacts with an immunological challenge affecting pain sensitivity in healthy subjects. This preliminary evidence, largely based on findings in healthy subjects, together with the methodological considerations discussed above, give way to future research on the role of immune-to-brain communication in FS symptom patients.

Prospects

It is now time for new studies aimed at demonstrating sensitized immune-to-brain communication in FS symptom patients. As suggested in Chapter 1, such studies should incorporate active manipulation of the immunological system, preferably in an experimental study setup. Below, a study design is proposed that resembles the design presented in Chapter 7, namely a placebo-controlled design with administration of an immunological challenge in both FS symptom patients and matched controls. Outcomes of (sensitized) immune-to-brain communication should be assessed both at the peripheral and central levels. Serum inflammatory markers can be measured as an indication of immunological activity at the peripheral level. Central immune system-related activity, however, can only be assessed indirectly by measurement of changes in behavior and mood (i.e. expressions of sickness behavior).

Specifically, a mixed 2 Groups (FS symptom patients, controls) x 2 Conditions (inflammation induction, placebo) design is proposed in which FS symptom patients are compared to persons without FS symptoms on their inflammatory and behavioral reactions to experimentally induced low-grade inflammation in a placebo controlled manner.

Selection of FS symptom participants

The theory of sensitized immune-to-brain communication is a general one, not aimed at explaining specific FS symptoms or syndromes. Thus, patients should

initially not be selected for inclusion based on symptom patterns but rather on overall symptom severity. In a later phase, studies on the theory of sensitized immune-to-brain communication could also investigate possible differences between groups with specific symptom patterns. Controls should be matched on age and gender, as FS symptom patients tend to be younger and more often female[2].

Induction of low-grade inflammation

Experimental induction of low-grade inflammation in humans is generally established through administration of *Salmonella typhi* vaccination or lipopolysaccharide (LPS) (see also Table 1 in Chapter 1, p. 11). LPS generally leads to a more pronounced inflammatory profile, accompanied by significant changes in mood and behavior in all subjects. *S. typhi* in contrast leads to less pronounced low-grade inflammation and is not always accompanied by changes in mood and behavior. Thus, the effects of *S. typhi* are more subtle and may be more suitable for detecting between group differences in inflammation-induced changes in behavior and mood because a ceiling effect would not occur. On the other hand, it is possible that a more severe immunological trigger is needed in order to find differences (physiologically, but also statistically, especially when small groups of subjects are compared, i.e., a design with a relatively low statistical power). Further studies are needed to find out which of these two manipulations is preferable to demonstrate sensitization of immune-to-brain communication.

Objective measurement of sickness behavior

Because symptom report is affected by several cognitive and affective mechanisms and FS symptom patients are known to over-report symptoms[3,4], it is recommended to include both subjective and objective measures of sickness behavior. Pain sensitivity is an appropriate more objective outcome measure of sickness behavior, as it has been demonstrated that this measure is affected by inflammation. Pain is also a common FS complaint. However, measures of both pain threshold and pain tolerance should be included and methodology for these

assessments should preferably be based on the recommendations presented in Chapter 3.

Inclusion of factors that may moderate inflammation-induced sickness behavior

We have shown NA to moderate (facilitate) inflammation-induced increases in pain sensitivity. Other possible candidates follow from animal models and include previous infections, severe life events, and chronic (on-going) stress[5-7]. These (moderating, facilitating, or cross-sensitising) factors, however, most likely differ across patients and future studies should focus on these individual differences. Sensitized immune-to-brain communication may not be an explanatory mechanism for all FS symptom patients and identification of moderating or cross-sensitising factors in humans will enable identification of subgroups of FS symptom patients whose symptoms can be explained by (different patterns of) sensitized immune-to-brain communication and/or different vulnerability patterns. Identification of these factors may also enable future screening for persons at risk, and they may serve as targets in future treatments.

Interpretation of study results

The extent to which low-grade inflammation (at the peripheral level) leads to the expression of sickness behavior is considered a marker of the sensitivity of immune-to-brain communication. Several results of future studies aimed at demonstrating a sensitized immune-to-brain communication in FS patients can be theorized: a) both FS symptom patients and controls show the same increase in peripheral inflammatory markers and the same amount of change in sickness behaviors, suggesting that immune-to-brain communication is not responsible for FS symptom expression; b) compared to controls, FS symptom patients show a more pronounced peripheral increase in inflammatory markers, accompanied by more pronounced changes in behavior and mood, suggesting that responses in peripheral immunity are sensitized in FS symptom patients; c) compared to controls, FS symptom patients show the same increase in peripheral inflammatory markers but show a more pronounced change in sickness behavior, suggesting

that immune-to-brain communication is sensitized specifically at a central level in FS symptom patients.

It is well possible that individual differences exist in the extent and the level (peripheral or central) of the sensitization. Studies aimed at unravelling these individual patterns using advanced statistical mixed model (multilevel random effects) techniques are therefore a necessary next step advancing from more classical statistical approaches that work with group averages.

Clinical implications

If sensitized immune-to-brain communication is indeed an underlying mechanism of FS symptoms, several treatment options are conceivable. First, there are some drugs known that inhibit immune-to-brain communication pathways in animals. For example, minocycline, a tetracycline antibiotic, is known to inhibit the central immune cells (microglia) that mediate immune-to-brain communication; it has already been shown that drugs like these can reduce or facilitate recovery from LPS-induced sickness behavior in animal models[8,9]. Second, if (subject-) specific psychosocial factors are identified that contribute to the sensitization of immune-to-brain communication, targeting these factors with, for example, psychotherapy may promote desensitization of the system or at least prevent further sensitization.

To conclude

Building on findings from animal models and the results on healthy human subjects reported here, this thesis serves as a prelude for new experimental placebo-controlled research designs specifically aimed at studying immune-to-brain communication in FS symptom patients, which is a promising perspective that is worth further testing.

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Nederlandse samenvatting

Dutch summary

Aanleiding voor het onderzoek

Onverklaarde lichamelijke klachten

Soms ervaren mensen lichamelijke klachten zonder dat een arts een medische oorzaak voor de klachten kan vinden. Deze klachten worden dan “medisch onverklaard” of “functioneel” genoemd. In de nieuwe Nederlandse multidisciplinaire richtlijn wordt gesproken van “somatisch onvoldoende verklaarde lichamelijke klachten”. De afkorting voor deze term, “SOLK”, zal gebruikt worden in deze samenvatting. De meest voorkomende SOLK zijn vermoeidheid- of pijngerelateerd. Psychische klachten zoals een depressieve stemming of angst komen ook vaak voor bij mensen met SOLK.

Wanneer SOLK langere tijd aanhouden wordt er soms een diagnose gesteld, gebaseerd op de klachten die iemand rapporteert. Bij aanhoudende ernstige vermoeidheidsklachten kan bijvoorbeeld het chronisch vermoeidheidsyndroom worden gediagnosticeerd, bij aanhoudende pijnklachten fibromyalgie en bij aanhoudende darmklachten het prikkelbare darmsyndroom. Vanuit de psychiatrie kan ook een diagnose worden gesteld zoals een somatoforme stoornis. Bij elke diagnose staat voorop dat er geen medische oorzaak voor de symptomen kan worden gevonden.

Immuun-breincommunicatie

Een theorie vanuit de psychoneuroimmunologie stelt dat SOLK misschien verklaard kunnen worden vanuit de immunologie. Deze theorie is gebaseerd op de overeenkomsten tussen SOLK en de klachten die ontstaan wanneer het immuunsysteem geactiveerd is. Bij aanwezigheid van bijvoorbeeld een virus of bacterie in het lichaam wordt het immuunsysteem actief. Dit gebeurt ook bij schade aan het lichaam, bijvoorbeeld een wond, en bij psychologische stress. Geactiveerde immuuncellen produceren zogeheten cytokinen, kleine boodschappercellen. Eén van de functies van die cytokinen is het communiceren van de toestand van het lichaam naar het brein en deze communicatie zet het brein aan tot veranderingen in gedrag en stemming. Voorbeelden van deze veranderingen zijn: vermoeidheid, lusteloosheid, pijn en een negatieve stemming. In het Engels worden deze veranderingen samengenomen in de term “sickness behavior”. In dit hoofdstuk zal de term “ziekteklachten” gebruikt worden om deze veranderingen in gedrag en stemming aan te duiden. Aangenomen wordt dat ziekteklachten een strategie zijn in het bestrijden van het virus of de bacterie: ze zorgen bijvoorbeeld voor een besparing van energie (die

dan gebruikt kan worden voor het opwekken van koorts) en ze voorkomen gedrag dat het lichaam blootstelt aan nieuwe infecties of schade aan het lichaam.

Immuun-breincommunicatie leidt dus tot ziekteklachten. De gelijkenis tussen deze klachten en SOLK is sterk. Zo zijn in beide condities pijn en vermoeidheid belangrijk en spelen stemmingsklachten een rol. Op basis hiervan is de theorie ontstaan dat SOLK misschien een chronische expressie van ziekteklachten zijn. Interessant hierbij is dat dieronderzoek laat zien dat de communicatie tussen het immuunsysteem en het brein ook gevoeliger, of gesensitiseerd, kan worden. Wanneer dieren worden blootgesteld aan herhaalde infecties of andere stressoren (psychologische stress of pijnprikkels), reageren ze op een nieuwe infectie of stressor met sterkere of langer durende ziekteklachten. Mogelijk kan de immuun-breincommunicatie in mensen ook gesensitiseerd raken en dit zou de ervaring van SOLK kunnen verklaren.

Experimenteel onderzoek waarbij immuun-breincommunicatie wordt geactiveerd en ziekteklachten wordt gemeten in mensen met SOLK zou hierover uitsluitsel kunnen geven. Dit proefschrift beschrijft een aantal studies die ingaan op methodologische aandachtspunten bij dit soort onderzoek. Daarnaast wordt een aantal studies gepresenteerd die de eerste bewijzen geven voor de mogelijkheid van een gesensitiseerde immuun-breincommunicatie als verklaring voor SOLK.

Methodologische aandachtspunten

Onderzoek naar SOLK-patiënten

In onderzoek naar SOLK wordt vaak gebruik gemaakt van bestaande diagnoses om mensen te selecteren voor deelname aan het onderzoek, bijvoorbeeld door het selecteren van alleen mensen met het chronisch vermoeidheidsyndroom. Hierbij wordt ervan uitgegaan dat er subgroepen van SOLK-patiënten bestaan die zich van elkaar onderscheiden door specifieke symptoompatronen. Het gebruik van deze diagnostische labels is echter al geruime tijd onderwerp van discussie en limiteert misschien ten onrechte de generaliseerbaarheid van uitkomsten van onderzoek. In **Hoofdstuk 2** wordt ingegaan op de vraag of er inderdaad subgroepen bestaan binnen SOLK. Hiervoor werden mensen met SOLK gevraagd van 47 lichamelijke klachten aan te geven hoeveel last ze gehad hadden van elke klacht in de twee weken voor het onderzoek. Door middel van een statistische techniek (*k-means clustering*) zijn ze daarna ingedeeld in groepen op basis van de scores die ze gaven aan de klachten. Hierbij zijn drie uitkomsten bekeken: een indeling in 2 groepen, een indeling in 3

groepen en een indeling in 4 groepen. In elke uitkomst bleken de groepen voornamelijk te verschillen in de mate waarin ze in totaal last hadden van lichamelijke klachten. De groepen verschilden echter niet in de soort klachten waar ze last van hadden. Alleen wanneer er drie of meer groepen waren gecreëerd, werden groepen zichtbaar die een ander klachtenpatroon liet zien. Deze groepen waren echter ook te onderscheiden op totale klachten-last en de statistische criteria waren het beste voor de oplossing met twee groepen. Uit Hoofdstuk 2 kan geconcludeerd worden dat mensen met SOLK niet per se geselecteerd hoeven worden op specifieke klachten, maar dat selectie ook plaats kan vinden op basis van de mate waarin mensen last hebben van SOLK.

Objectieve maten van ziekteklachten

Onderzoek naar ziekteklachten bij mensen heeft tot nu toe voornamelijk gebruik gemaakt van vragenlijsten. Mensen werd hierbij gevraagd zelf aan te geven hoe ze zich voelden en dit maakt de metingen subjectief. Objectieve maten worden, in vergelijking met subjectieve maten, niet of minder beïnvloed door factoren als stemming, geheugen, of de neiging tot overrapportage en hebben dus de voorkeur. **Hoofdstuk 3** betreft een studie over de methodologie van een objectieve meting van pijnsensitiviteit. In deze studie werd gebruik gemaakt van druk als pijnlijke prikkel: tijdens een meting werd met een rubberen dopje van ongeveer 1 cm^2 druk op de huid of de nagel uitgeoefend. De druk werd langzaam opgevoerd tot de deelnemer aangaf het gewenste niveau bereikt te hebben. Uit de studie bleek onder andere dat de pijndrempel (druk waarbij voor het eerst pijn wordt ervaren) en de pijntolerantie (druk waarbij de pijn ondraaglijk wordt) aparte aspecten van pijnsensitiviteit zijn en dat dus beiden gemeten zouden moeten worden wanneer pijnsensitiviteit bestudeerd wordt. Daarnaast konden op basis van de resultaten aanbevelingen gedaan worden ten aanzien van het aantal metingen per lichaamspunt en analyses.

Hoofdstuk 4 gaat in op een mogelijke objectieve meting van cognitieve vermoeidheid, namelijk de combinatie van prestatie op een cognitieve taak en de inspanning tijdens de taak. Cognitieve inspanning werd hierbij gemeten door middel van pupillometrie. Pupillometrie betreft de meting van de grootte van de oogpupil; de oogpupil wordt groter tijdens mentale inspanning en de verandering in grootte kan worden beschouwd als een maat voor de inspanning die wordt geleverd. In de twee studies die in Hoofdstuk 4 beschreven staan werd geen bewijs gevonden dat prestatie en inspanning een reflectie zijn van cognitieve vermoeidheid. Door een aantal limitaties

in beide studies echter, kunnen we op dit moment niet met zekerheid zeggen dat prestatie en inspanning geen reflectie zijn van cognitieve vermoeidheid. Hiervoor is verder onderzoek nodig.

Eerste studies over een mogelijk gesensitiseerde immuun-brein communicatie in SOLK

In **Hoofdstuk 5** wordt een studie beschreven waaruit blijkt dat pijnsensitiviteit gemeten in de endeldarm (een maat voor viscerale pijn; binnenin het lichaam) kan worden voorspeld door zowel stress-gerelateerde factoren (angst, cortisollevels) als immunologische factoren (buikklachten gerelateerd aan immunologische activiteit). Verhoogde pijnsensitiviteit is een belangrijk aspect van ziekteklachten en deze studie laat zien dat psychologische en immunologische stressoren allebei invloed hebben op de mechanismen die leiden tot pijnsensitiviteit. Dit impliceert dat gevoeligheid kan optreden wanneer meerdere stressoren aanwezig zijn.

Hoofdstuk 6 gaat in op de vraag of de voorwaarden voor een gesensitiseerde immuun-brein communicatie aanwezig zijn in SOLK-patiënten. Dieronderzoek heeft aangetoond dat sensitisatie kan optreden bij blootstelling aan meerdere stressoren of infecties. SOLK-patiënten lijken wel vaker vroege jeugdtrauma's te hebben meegemaakt en in het algemeen meer stress te ervaren, maar er is weinig bekend over of ze ook meer infecties hebben gehad. In de studie beschreven in Hoofdstuk 6 is een groep patiënten met een somatoforme stoornis (gekarakteriseerd door meerdere, chronische SOLK) vergeleken met een controlegroep op het aantal infecties zoals geregistreerd door de huisarts. Alleen registraties van infecties *voorafgaand* aan de diagnose van de somatoforme stoornis (of een gelijke datum voor de persoon in de controlegroep) zijn geteld. Uit de studie bleek dat de groep met somatoforme stoornissen inderdaad meer infectieregistraties had dan de controlegroep. Deze bevinding bevestigt dat de voorwaarden voor een gesensitiseerde immuun-breincommunicatie in mensen met SOLK aanwezig zijn.

Er is nog weinig onderzoek gedaan naar sensitiviteit van immuun-breincommunicatie in mensen. **Hoofdstuk 7** beschrijft een studie waarin is onderzocht of stress, of persoonlijke eigenschappen die mogelijk leiden tot stressgevoeligheid, gerelateerd zijn aan ziekteklachten in mensen. Hiervoor werden gezonde jonge vrouwen geïnjecteerd met een vaccinatie of een zoutoplossing (placebo) op twee

verschillende testdagen. Pijnsensitiviteit (zoals beschreven in Hoofdstuk 2) werd op beide dagen gemeten voorafgaand aan de injectie en zes uur na de injectie. De vaccinatie leidde tot immunologische activiteit en dus tot immuun-breincommunicatie. De zoutoplossing leidde niet tot immunologische activiteit. De studie liet zien dat in mensen die informatie vaker negatief interpreteren en een sterkere emotionele reactie hebben op negatieve gebeurtenissen (hoge negatieve affectiviteit), vaccinatie leidde tot een verhoogde pijnsensitiviteit. Pijnsensitiviteit veranderde niet in de placeboconditie. Negatieve affectiviteit zou mogelijk kunnen leiden tot het ervaren van meer, of meer negatieve, stressoren. Door het ervaren van meer stressoren zou bij mensen met een hoge negatieve affectiviteit de immuun-breincommunicatie gesensitiseerd kunnen raken. Uit de studie kan worden geconcludeerd dat negatieve affectiviteit mogelijk de door vaccinatie opgewekte immuun-breincommunicatie versterkt zodat er veranderingen in pijnsensitiviteit optreden.

Aanbevelingen voor toekomstig onderzoek

De in dit proefschrift gepresenteerde studies kunnen worden gebruikt om onderzoek naar immuun-brein communicatie in mensen met SOLK op te zetten. In **Hoofdstuk 8** wordt een onderzoeksvoorstel beschreven op basis van deze bevindingen. Hierin staat centraal dat het immuunsysteem geactiveerd moet worden door middel van bijvoorbeeld een vaccinatie. Ziekteklachten worden gemeten voor en na de vaccinatie op de ene dag en voor en na injectie met een zoutoplossing op een andere dag. Op deze manier kunnen veranderingen in ziekteklachten door de vaccinatie vergeleken worden met veranderingen door placebo. Door mensen met SOLK en mensen zonder SOLK te meten, kan de verandering in ziekteklachten worden vergeleken tussen deze twee groepen.

Met betrekking tot het selecteren van SOLK-patiënten, laat Hoofdstuk 2 van dit proefschrift zien dat selectie niet gebaseerd hoeft te zijn op specifieke klachten. Aangezien immuun-breincommunicatie leidt tot een verscheidenheid aan klachten, is het wenselijk om mensen met verschillende SOLK te selecteren.

In de studie zouden verschillende ziekteklachten gemeten moeten worden, liefst op een objectieve manier, dus zo min mogelijk met vragenlijsten. Pijnsensitiviteit kan gemeten worden volgens de aanbevelingen in Hoofdstuk 3. Voor een objectieve maat van vermoeidheid is meer onderzoek nodig.

Om een beeld te krijgen van stress-gerelateerde factoren die de immuun-brein communicatie gesensitiseerd zouden kunnen hebben, zouden verschillende van zulke factoren, zoals negatieve affectiviteit (Hoofdstuk 7) en de hoeveelheid doorgemaakte infecties (Hoofdstuk 6), gemeten kunnen worden voorafgaand aan de injecties.

Conclusie

De voornamelijk op dieronderzoek gebaseerde hypothese van SOLK verklaard vanuit immuun-breincommunicatie zou getest moeten worden in mensen. Dit proefschrift dient als een introductie voor dit onderzoek. Methodologische keuzes aangaande selectie van SOLK-patiënten en objectieve metingen van ziektegedrag kunnen worden gebaseerd op de bevindingen gepresenteerd in de eerste hoofdstukken van dit proefschrift. Eerste aanwijzingen voor de validiteit van de hypothese zijn gepresenteerd in het tweede deel van proefschrift en dienen als onderbouwing van verder onderzoek. Nu is het tijd voor werkelijk onderzoek naar immuun-breincommunicatie in SOLK-patiënten.

Dankwoord

Acknowledgements

Eindelijk! Af! De afgelopen vijf jaar waren waarschijnlijk zeer representatief voor wat elke AiO meemaakt: veel ups en downs en een eindsprint waar je maar (cognitief) moe van wordt. Net als elke AiO ben ik dan ook erg blij met de mensen die me begeleid hebben, me gesteund hebben en me af en toe afgeleid hebben.

Lorenz van Doornen, ik ben voor ons allebei blij dat ik je nog even van de geraniums heb weggehouden. Ik heb met jou als promotor een enorme vrijheid ervaren zonder het gevoel te krijgen te verdrinken. Door de vrijheid die je me gaf heb ik erg veel geleerd. Over onderzoek doen natuurlijk, maar ook over omgaan met teleurstellingen en op eigen benen durven te staan. Dank je wel daarvoor.

Jan Houtveen, ik had me geen betere begeleider dan jou kunnen wensen! Dank je wel dat je altijd tijd voor me had, met me mee wilde denken, het geduld had me van alles en nog wat te leren (van solderen tot plaatjes print klaar maken en oja, ook nog een berg onderzoeksvaardigheden) en me dan ook nog op je bruiloftsfeest wilde hebben. Fijn dat ik altijd bij je binnen kon lopen als er weer eens structuur aangebracht moest worden in een gedachtelijn of analyse. Ik heb erg veel geleerd van je altijd uitgebreide feedback op mijn stukken en dat heeft me het zelfvertrouwen gegeven om het nu zonder jouw begeleiding te gaan proberen.

Cobi Heijnen, jammer genoeg is het project een kant opgegaan waarbij jouw kennis en expertise minder nodig waren. Ik ben erg blij dat ik vanaf september alsnog van je mag gaan leren!

Spending some time in other research groups is certainly an enlightening experience. Therefore, Sigrid Elsenbruch, thank you for the opportunity to work within your research group. I learned a lot and got a nice paper out of it too! Sven, Julia, and Joswin, thank you for making my stay in Germany interesting and fun.

Jos Bosch, zonder jou was het laatste hoofdstuk van mijn proefschrift er nooit gekomen. Dank je wel dat je het mogelijk hebt gemaakt voor me om een studie in Birmingham te draaien. Jet Veldhuizen van Zanten, dank je wel voor je geweldige begeleiding van mij en Lotte in Birmingham. Hoewel ook deze studie niet precies ging

zoals ik had gewild (maar wie verzint er nou dat je vaccinatiebatch wordt teruggehaald door de fabrikant!), is het toch een mooie studie geworden met alvast één mooie paper.

Ninke, ik heb een superleuke tijd gehad met jou op één werkkamer. Ik zal de bijna automatische "hoe is t's" elke ochtend, de eigen baksels, de feestelijke , troostende, of gewoon-omdat-het-vrijdag-is appelflappen en je ongebreidelde enthousiasme enorm gaan missen! Ik ben blij dat we de deze zomer toch nog gewoon een kamer hebben kunnen delen én dat je mijn paranimf wilt zijn. Zonder jou zou de hele emotionele kant van de promotie waarschijnlijk voor een groot deel aan me voorbij gaan ☺. Heel veel succes met jouw laatste loodjes, ik ga mijn best doen je vanuit Houston net zo goed te ondersteunen als je mij hebt gesupport.

Maarten, aan sommige mensen moet ik even wennen voor ik ze aardig ga vinden. Het duurde even (sorry daarvoor), maar ik ben je droge humor, je kijk op de wereld en je enthousiasme voor statistische gesprekken enorm gaan waarderen en zal dat gaan missen in Houston. Dank je wel dat je mijn paranimf wilt zijn.

Marianne, Karin, Miriam en Eliane, fijn dat ik altijd kon binnen lopen voor een praatje en dat jullie regelmatig bij ons binnenliepen. Laten we proberen die etentjes voort te blijven zetten!

Cécile, je was er wat minder vaak dan andere collega's, maar als je er was, was het gezellig om een kamer met je te delen. Succes met het afronden van je proefschrift. Die laatste hoofdstukken gaan echt een stuk sneller dan de eerste!

Als laatste en dus enig overgebleven AiO-telg van Lorenz' onderzoeksgroep heb ik me wel eens op een eilandje gevoeld. Dankzij alle gezellige lunches, AiO-bijeenkomsten, borrels en etentjes bleef dit gevoel beperkt tot het onderzoeksonderwerp, dank dus aan iedereen voor alle gezelligheid.

En dan was er ook nog een leven buiten de uni…

Elizabet, mooi dat we al zo lang bevriend zijn! Ik vind het altijd gezellig om je te zien en te horen wat je nu weer allemaal aan het doen bent. Ik heb enorm veel bewondering voor wat jij allemaal doet en hoe je telkens weer durft te gaan voor wat je wilt.

Remco, wat was het soms lastig om een kopje koffie in te plannen! ik ben blij dat we het toch elke keer weer voor elkaar kregen. Hopelijk kunnen we wat vinden op de grotere afstand straks (koffie tijdens het skypen?).

Bregje, lief zusje! Dit heb ik de afgelopen vijf jaar nou gedaan ☺. Ik ben blij dat je doet waar je van geniet. Ik zal je mooie stem missen in Houston, dus stuur me maar vaak een demo.

Monica, fijn dat je er bent in het Lacourtse leven! Ik vind het altijd leuk om naar Nijmegen af te reizen voor een gezellig etentje met (soms te veel) lekkere wijn en dat komt zeker mede door jou.

Mama en papa, dank jullie wel voor jullie steun en aanmoediging in zo'n beetje alles. Ik weet dat jullie trots op me zijn en dat betekent enorm veel voor me.

Curriculum Vitae

Tamara Lacourt was born in Ede, the Netherlands, at October 30, 1979. She followed her secondary education at Titus Brandsma Lyceum in Oss, graduating in 1998. After trying out several different studies and jobs, she decided on a study in Psychology at Utrecht University in 2003, combined with a career in the food service industry. This resulted in a Bachelor degree in 2006 and the ability to serve food at the speed of light. In 2008, she obtained her Master degree cum laude in Health Psychology at Leiden University.

As her interest during her studies were mainly focused on biological aspects of psychology and as writing her master thesis had ignited a passion for research, doing a PhD in biopsychology seemed a good next step. Thus, in 2008, she started on a PhD project concerning immune-to-brain communication in persons with medically unexplained somatic symptoms under the supervision of Prof. Lorenz van Doornen and Dr. Jan Houtveen and with support from Prof. Cobi Heijnen. During this project, she spent some time at the University of Duisburg-Essen under the supervision of Prof. Sigrid Elsenbruch and at the University of Birmingham, where she worked with Dr. Jet Veldhuijzen van Zanten.

Despite some setbacks inherent to all PhD projects, this project has culminated in a PhD thesis which will be defended on October 18, 2013. In the meanwhile, she will start as a post-doctoral researcher in September 2013 at the Department of Symptom Research at MD Anderson in Houston, USA, under the supervision of Prof. Charles Cleeland and Prof. Cobi Heijnen.

Appendixes

Appendix A

Somatic complaints questionnaire described in **Chapter 2**.

*In this questionnaire, you are asked to what extent you were experiencing somatic symptoms. Please indicate **for every symptom in the list** the answer that is most applicable for you. For every symptom, we want to know how you felt during **the last week, including today**.*

To what extend did you experience:

	not	a bit	quite a bit	quite a lot	highly
headache	0	0	0	0	0
dizziness	0	0	0	0	0
fainting	0	0	0	0	0
nausea	0	0	0	0	0
rustling sound in ears	0	0	0	0	0
confusion or feelings of unreality	0	0	0	0	0
upset stomach	0	0	0	0	0
abdominal pain or stomach pain	0	0	0	0	0
bowel cramps	0	0	0	0	0
bloated stomach	0	0	0	0	0
feeling low on energy	0	0	0	0	0
feeling tired	0	0	0	0	0
feeling exhausted	0	0	0	0	0
feeling physically weak	0	0	0	0	0
not feeling fit	0	0	0	0	0
chest pain	0	0	0	0	0
tightness around the chest	0	0	0	0	0
rapid heartbeat	0	0	0	0	0
pounding heart	0	0	0	0	0
irregular heart beat	0	0	0	0	0
painful stings in the heart area	0	0	0	0	0
feelings of dyspnea	0	0	0	0	0

shortness of breath	0	0	0	0	0
inability to take a deep breath	0	0	0	0	0
sudden fast or deep breathing	0	0	0	0	0
breathlessness	0	0	0	0	0
muscle pain	0	0	0	0	0
pain in bones	0	0	0	0	0
back pain	0	0	0	0	0
pain in neck	0	0	0	0	0
feelings of muscle weakness	0	0	0	0	0
stiffness of fingers, arms, or legs	0	0	0	0	0
trembling of hands, arms, or legs	0	0	0	0	0
excessive sweating	0	0	0	0	0
hot or cold flashes	0	0	0	0	0
tingling feeling in fingers, arms, or legs	0	0	0	0	0
numb feeling somewhere in body	0	0	0	0	0
dry mouth	0	0	0	0	0
lump in throat	0	0	0	0	0
having trouble swallowing	0	0	0	0	0
sore throat	0	0	0	0	0
difficulty concentrating	0	0	0	0	0
forgetfulness	0	0	0	0	0
having trouble paying attention	0	0	0	0	0
unclear or foggy thoughts	0	0	0	0	0
distracting thoughts	0	0	0	0	0
pain in joints	0	0	0	0	0

Appendix B

Overview of used DSM-IV and ICPC codes for in- and exclusion criteria for the study described in **Chapter 6**.

DSM-IV codes in PCR-MN

Inclusion in SD group if registration in PCR-MN for:

- 300.82 (Undifferentiated somatoform disorder);
- 300.81 (Somatisation disorder);
- 307.80 (Pain disorder associated with psychological factors);
- 307.89 (Pain disorder associated with both psychological factors and a general medical condition).

Exclusion from Control group if registration in PCR-MN for:

- 300.82 (Undifferentiated somatoform disorder;
- 300.81 (Somatisation disorder);
- 307.80 (Pain disorder associated with psychological factors);
- 307.89 (Pain disorder associated with both psychological factors and a general medical condition);
- 300.11 (Conversion disorder).

Exclusion from all samples if registration in PCR-MN for:

- 295.40 (Schizophreniform disorder);
- 295.71 (Schizoaffective disorder);
- 297.1 (Delusional disorder);
- 298.8 (Brief psychotic disorder);
- 297.3 (Shared psychotic disorder);
- 293.8 (Psychotic disorder due to medical condition);
- 298.9 (Psychotic disorder not otherwise specified);
- 296.0 (Bipolar I disorder, single manic episode);
- 296.4 (Bipolar I disorder, most recent episode manic);
- 296.5 (Bipolar I disorder, most recent episode depressed);
- 296.6 (Bipolar I disorder, most recent episode mixed);
- 296.7 (Bipolar I disorder, most recent episode unspecified).

ICPC codes in JGPN

Exclusion from Control group in case of registration in JGPN for:

- D93.00 (Irritable Bowel Syndrome);
- P75.00 (Hysteria/Hypochondria);
- A04.01 (Chronic fatigue syndrome);
- L18.01 (Fibromyalgia).

Or three registrations within six months (and without being followed by registration of a medical condition within 30 days of the registration) for:

- A04.00 (Tiredness/weakness);
- A05.00 (General deterioration);
- L18.00 (Muscle pain);
- L19.00 (Symptoms of several or not specified muscles).