MD(RES)

NEUROPHYSIOLOGICAL, PSYCHOLOGICAL AND IMMUNOLOGICAL EVALUATION OF IRRITABLE BOWEL SYNDROME

Dr Dominic Bullas

Division of Surgery, Oncology, Reproductive Biology and Anaesthetics

Imperial College London

January 2012

DECLARATION OF ORIGINALITY

Title of thesis: **Neurophysiological, psychological and immunological evaluation of Irritable Bowel Syndrome**

This body of work is original and authentic. This thesis was written by myself. All citations have been formally referenced. Although the work is my own, it is a product of the help and expertise of many collaborators. I was assisted in procuring the cortical evoked potentials by Salma Gurmany; in the processing and interpretation of the cortical evoked potentials by Dr Anthony Hobson; in the development of the protocol for cytokine analysis by Dr David Bulmer. Dr Laurens Kruidenier taught me the laboratory craft needed to process the cytokine samples. Kirsty Hicks provided statistical support. My academic supervisors, Dr Arebi and Professor Atkin, guided me in the interrogation of the dataset and oversaw the development of the thesis and its associated publications. Imperial College provided funding for the study through the aADI educational grant from GlaxoSmithKline.

ABSTRACT

Name of candidate: **Dr Dominic Bullas**

Title of thesis: **Neurophysiological, psychological and immunological evaluation of Irritable Bowel Syndrome**

Irritable Bowel Syndrome (IBS) is a common gastroenterological disorder characterised by recurrent abdominal pain and a change in bowel habit. Diagnosis is made with reference to clinical symptomatology and the exclusion of other competing disorders. As such there is no reliable biomarker in the clinical field to prove or disprove the diagnosis. This has obvious implications for the treatment of the syndrome. Recent research has targeted the immunology of IBS to see if changes in cytokine expression support the concept of a chronic low-level inflammatory state. IBS has a strong association with psychological comorbidity. Visceral hypersensitivity is also a hallmark of the disease.

This study was constructed to compare the psychological profiles of patients with IBS against a control group; to use cortical evoked potentials to observe the cortical representation of an electrical rectal stimulus to see if changes in amplitude or latency might be suggestive of enhanced afferent nerve transmission; and to use cytokine analysis of stimulated and un-stimulated peripheral blood mononuclear cells to study the inflammatory response.

ACKNOWLEDGEMENTS

Dr Anthony Hobson

Dr Arebi

Professor Wendy Atkin

Professor Michael Kamm

Salma Gurmany

Dr Laurens Kruidenier

Dr David Bulmer

Dr George Dukes

ABBREVIATIONS

CEP	Cortical evoked potential
EP	Evoked potential
EPQ-R	Eysenck Personality Questionnaire- Revised edition
FGIDs	Functional Gastrointestinal Disorders
LPS	Lipopolysaccharide
HAD	Hospital Anxiety and Depression Index
HPA Axis	Hypothalamic-Pituitary-Adrenal Axis
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
IBS-C	Constipation-predominant IBS
IBS-D	Diarrhoea-predominance IBS
IBS-M	Mixed-type IBS (i.e. alternating stool consistency)
IBS-U	Unsubtyped IBS
IBS-IS	Irritable Bowel Syndrome Impact Scale
IBSSS	Irritable Bowel Severity Scoring System
IFN	Interferon
IBAM	Idiopathic Bile Acid Malabsorption
IL	Interleukin
MEG	Magnetoencephalography
PBMCs	Peripheral Blood Mononuclear Cells
PHQ-15	Patient Health Questionnaire 15

PT	Pain threshold
SSTAI	Spielberger State Trait Anxiety Inventory
ST	Sensory threshold
TNF	Tissue Necrosis Factor
TT	Test threshold
VDVAS	Visual Descriptor Visual Analogue Scale
VSI	Visceral Sensitivity Index
WI	Whiteley Index

BLANK PAGE

BLANK PAGE

TABLE OF CONTENTS

Declaration of Originality 2

Abstract 3

Acknowledgements 4

Abbreviations 5

CHAPTER ONE, Section 1: Clinical Presentation Of Irritable Bowel Syndrome 17

Epidemiology and Social Impact of Irritable Bowel Syndrome 17

Epidemiology 17

Prevalence 17

Age 17

Incidence 18

Gender 18

Culture 19

Social Impact 20

Quality Of Life 20

Absenteeism 20

Health-seeking Behaviour 21

Surgery 21

Suicide 21

Direct Costs of Irritable Bowel Syndrome 22

Indirect Costs of Irritable Bowel Syndrome 22

Diagnosis of Irritable Bowel Syndrome 23

Clinical Presentation 23

The Rome Committee 23

Manning Criteria 23

Kruis Criteria 24

Rome Criteria 26

Symptom Variability 32

IBS Sub-types 32

Natural history 32

Associated Functional Gastrointestinal Disorders 33

Associated non-gastrointestinal non-psychiatric symptomatology 34

Psychiatric Comorbidity 34

Life events 35

Abuse 36

Mood and Anxiety Disorders 37

Personality disorders 37

Differential Diagnosis *38*
Food Allergy and Intolerance 39
Lactose intolerance 39
Post-Infectious IBS 39
Antibiotic-associated Diarrhoea 40
Primary Idiopathic Bile Acid Malabsorption 40
Endocrine Causes of Diarrhoea 40
Other Forms of Colitis 40
Intestinal Pseudo-Obstruction 41
Hirschsprung's Disease 41
Excluding the Differential Diagnosis *42*
History 42
Examination 42
Primary Care 42
Secondary Care 43
Global approach to management 43
Treating Irritable Bowel Syndrome *45*
Conservative Management 45
Self-Management 46
Multidisciplinary Team 46
Diet and Exercise 46
Complementary and Alternative Medicine 48
Medical Therapy 49
Anti-motility drugs 49
Antispasmodics 49
Probiotics 49
Antidepressants 50
Psychological Therapy 50
Self-Help Groups 50
Relaxation Therapy 51
Biofeedback 51
Hypnotherapy 51
Cognitive Behavioural Therapy 52
Psychodynamic Interpersonal Psychotherapy 53

CHAPTER ONE, Section 2: Pathogenesis of Irritable Bowel Syndrome **55**
Psychopathology *55*
Abnormal Illness Behaviour 57
Biopsychosocial Model of FGIDs 60
Micro-inflammation *61*
Post-Infectious Irritable Bowel Syndrome 61
Figure 1.2.1 Incidence of Post-infectious Irritable Bowel Syndrome 63
Inflammation in Irritable Bowel Syndrome 64
Cytokines 64
Mucosal Permeability 66
Visceral Hypersensitivity *68*
Autonomic Dysfunction *69*
Hypothalamic-Pituitary Axis *69*
CHAPTER ONE, Section 3: Research Instruments used in the study protocols **70**
Clinical Scoring Systems *70*
Irritable Bowel Severity Scoring System 70
Verbal Descriptor Visual Analogue Scale 71
Psychometric Testing *72*
Hospital Anxiety and Disease Scale 72
Eysenck Personality Questionnaire 73
Whiteley Index 77
Patient Health Questionnaire 15 77
Visceral Sensitivity Index 78
Figure 1.3.1 Role of Stress in the development and modulation of IBS 78
Spielberger State Trait Anxiety Index 79
Quality Of Life Scores *80*
Irritable Bowel Syndrome Impact Scale 80
Figure 1.3.2 Irritable Bowel Syndrome Impact Scale 83
Neurophysiology *82*
Cortical Evoked Potentials 82
Figure 1.3.3 Endo-rectal evoked response 83
Figure 1.3.4. A Cortical Evoked Potential recorded after electrical stimulation in a Control 85
Figure 1.3.5. Rectal evoked potentials using varying stimulus intensities 86
Figure 1.3.6. Oesophageal evoked potentials during anticipatory stimulation 87

CHAPTER ONE, Section 4: Hypothesis & Aims **88**
Hypothesis *88*
Aims *88*
Psychological Questionnaires 88
Cortical Evoked Potentials 89
Cytokine Analysis 89
CHAPTER TWO, Methodology **90**
Study Design *90*
Inclusion Criteria 90
Exclusion Criteria 90
Endpoints 91
Assessment of Psychological Factors 91
Assessment of Clinical Severity 91
Sensory and pain thresholds 91
CEP Analysis 91
Immunological and Endocrine Endpoints 91
Patient Flow Chart 92
Psychological Assessment 93
Cortical Evoked Potential Protocol 94
Rectal Sensitivity Testing 94
Cortical Evoked Potential Testing 95
Cytokine Protocol 97
Isolation and Stimulation of Peripheral Blood Mononuclear Cells 97
Cytokine Assay 98
Cytokine Analysis 99
Figure 2.1 Preparation of the calibration curve 100
Figure 2.2 Ninety-six well plate setup for MSD-Plex Cytokine Array 101
CHAPTER THREE, Section One: CEP Study Results **104**
Table 3.1.1a Study Demographics 104
IBS Subtypes 105
Figure 3.1.1 IBS subtypes for the CEP study 105
Table 3.1.1b CEP Study Completion 105
Study Power 106
Table 3.1.1c Sensory Thresholds and Pain Thresholds to Oesophageal Stimulation 106
Statistics 106

Psychological Analysis *107*
Day 1 Psychological Tests 108
Figure 3.1.2 Eysenck Personality Questionnaire- Revised: Psychoticism 108
Figure 3.1.3 Eysenck Personality Questionnaire- Revised: Extraversion 109
Figure 3.1.4 Eysenck Personality Questionnaire- Revised: Neuroticism 110
Figure 3.1.5 Eysenck Personality Questionnaire- Revised: Lie 111
Figure 3.1.6 Eysenck Personality Questionnaire- Revised: Addiction 112
Figure 3.1.7 Eysenck Personality Questionnaire- Revised: Criminality 113
Figure 3.1.8 Visceral Sensitivity Index 114
Figure 3.1.9 Patient Health Questionnaire 15 115
Figure 3.1.10 Whiteley Index 116
Day 1 to 3 Psychological Tests 117
Figure 3.1.11 Hospital Anxiety and Depression: Anxiety Score 118
Figure 3.1.12 Hospital Anxiety and Depression: Depression Score 119
Figure 3.1.13 Spielberger State Trait Anxiety Inventory: State Anxiety 120
Figure 3.1.14 Spielberger State Trait Anxiety Inventory: Trait Anxiety 121
Figure 3.1.15 Visual Descriptor Visual Analogue Scale: Unpleasantness of Symptoms 122
Figure 3.1.16 Visual Descriptor Visual Analogue Scale: Intensity of Symptoms 123
Anorectal Sensitivity *124*
Figure 3.1.17 Sensory Thresholds 125
Figure 3.1.18 Pain Thresholds 126
Cortical Evoked Potentials *127*
Figure 3.1.19 A CEP recorded after a single stimulation from a Control subject 127
Figure 3.1.20 A CEP recorded after 1 cycle of 50 stimulations from a Control subject 127
Figure 3.1.21 CEPs recorded over 4 cycles of 50 electrical stimulations from a Control subject 128
Figure 3.1.22 Averaged CEPs recorded over 3 separate days from a Control subject 128
Intra-day and inter-day variability of Cortical evoked potentials in the Control group 129
Table 3.1.22b One Way ANOVA of the mean difference of anorectal sensitivities, CEP peak latencies and CEP peak amplitudes between Day 2 and Day 1 in the Control group 129
Table 3.1.22c One Way ANOVA of the mean difference of anorectal sensitivities, CEP peak latencies and CEP peak amplitudes between Day 3 and Day 1 in the Control group 130
Table 3.1.22d One Way ANOVA of the mean difference of anorectal sensitivities, CEP peak latencies and CEP peak amplitudes between Day 3 and Day 2 in the Control group 130
Figure 3.1.23 Peak 1 Latency over 3 days 131
Figure 3.1.24 Peak 2 Latency over 3 days 132
Figure 3.1.25 Peak 3 Latency over 3 days 133
Figure 3.1.26 Peak 4 Latency over 3 days 134
Figure 3.1.27 Peak 5 Latency over 3 days 135
Table 3.1.27a Peak 5 Latency over 3 days 135

Table 3.1.27b Summary of CEP Peak Latency Data 136
Figure 3.1.28 Peak 1 Amplitude 137
Figure 3.1.29 Peak 2 Amplitude 138
Figure 3.1.30 Peak 3 Amplitude 139
Figure 3.1.31 Peak 4 Amplitude 140
Correlations of the CEP Study Data 142
Table 3.1.32 Spearman Correlation Coefficients of the IBS Group 142
CHAPTER THREE, Section Two: Cytokine Study Results 144
Table 3.2.1 IBS/Control Demographics 144
Figure 3.2.1 IBS subtypes for the Cytokine study 144
Psychological Data 145
Figure 3.2.2 Irritable Bowel Severity Scoring System 145
Figure 3.2.3 Irritable Bowel Syndrome Impact Scale 146
Pro-Inflammatory Cytokine Data 147
Figure 3.2.4 TNF-α release from LPS-stimulated PBMCs 148
Figure 3.2.5 IFN-γ release from LPS-stimulated PBMCs 149
Figure 3.2.6 IL-1-βrelease from LPS-stimulated PBMCs 150
Figure 3.2.7 IL-2 release from LPS-stimulated PBMCs 151
Figure 3.2.8 IL-6 release from LPS-stimulated PBMCs 152
Figure 3.2.9 IL-8 release from LPS-stimulated PBMCs 153
Figure 3.2.10 IL-12 release from LPS-stimulated PBMCs 154
Anti-Inflammatory Cytokine Data 155
Figure 3.2.11 IL-4 release from LPS-stimulated PBMCs 155
Figure 3.2.12 IL-5 release from LPS-stimulated PBMCs 156
Figure 3.2.13 IL-10 release from LPS-stimulated PBMCs 157
Figure 3.2.14 IL-13 release from LPS-stimulated PBMCs 158
Correlations of the Cytokine Data 159
Table 3.2.15 Spearman Correlation Coefficients of the IBS Group 159
Table 3.2.16 Summary of the Cytokine Correlations 162
Figure 3.2.15 Selected Whole Group Correlations 162

CHAPTER FOUR: Discussion **163**

Psychology *163*

Neurophysiology *172*

Figure 4.1 Patient Stratification using Pain Threshold and Peak 1 Latency 176

Figure 4.2 Identifying the IBS Subgroups using Pain Threshold and Peak 1 Latency 177

Figure 4.4 Neuroanatomy of the Ascending Visceral Afferents 183

Immunology *188*

Figure 4.5 TNF-α Production from Controls and IBS patients with and without LPS stimulation (Liebgrets et al.) 189

Figure 4.6 TNF-α Production from Controls and IBS patients with and without LPS stimulation 190

Figure 4.7 IL-1 β Production from Controls and IBS patients with and without LPS stimulation (Liebgrets et al.) 190

Figure 4.8 IL-1 β Production from Controls and IBS patients with and without LPS stimulation 191

Figure 4.9 IL-6 Production from Controls and IBS patients with and without LPS stimulation (Liebgrets et al.) 191

Figure 4.10 IL-6 Production from Controls and IBS patients with and without LPS stimulation 192

Figure 4.11 Correlation of E coli LPS-induced TNF-α production and HAD-Anxiety Scores (Liebregts et al.) 196

Figure 4.12 The T_{H17} Pathway 201

Final Conclusion *204*

APPENDIX **204**

British Society of Gastroenterology Poster, 13.03.08 205

Clinical phenotyping of Irritable Bowel Syndrome in a prospective controlled trial using psychological profiling and cortical evoked action potentials

Neurogastroenterology & Motility Poster, 07.11.08 206

Test re-test reproducibility of rectal cortical evoked potentials in patients with Irritable Bowel Syndrome

Neurogastroenterology & Motility Poster, 07.11.08 207

The clinical expression of psychological comorbidity over time in Irritable Bowel Syndrome

Neurogastroenterology & Motility Poster, 07.11.08 208

Is rectal afferent transmission altered in patients with Irritable Bowel Syndrome?

Neurogastroenterology & Motility Poster, 07.11.08 209

Neurophysiological assessment of rectal afferent sensitivity in patients with Irritable Bowel Syndrome defines distinct clinical phenotypes.

British Society of Gastroenterology Poster, 25.03.09 210

Objective assessment of rectal afferent sensitivity and primary cortical processing in patients with Irritable Bowel Syndrome

Neurogastroenterology & Motility Poster, 28.08.09 211

Psychological comorbidity and symptom intensity do not correlate with rectal afferent sensitivity as determined by objective neurophysiological assessment in Irritable Bowel Syndrome.

UEGW/WCOG Gastro 2009 Poster, 23.11.09, London 212

High depression and anxiety scores in a systematic review of Psychological Questionnaires in Irritable Bowel Syndrome

British Society of Gastroenterology Poster, Liverpool ACC, 25th March 2010 213

Stress-induced immunomodulation in Irritable Bowel Syndrome suppresses TH1 and TH2 cytokines

AGA Institute, Digestive Disease Week, New Orleans, LA, May 4th 2010 214

Suppression of TH1 and TH2 cytokines in patients with Irritable Bowel Syndrome-evidence of a chronic stress immunophenotype?

Am J Physiol Gastrointest Liver Physiol. February 24th 2011 215

Distinct Neurophysiological Profiles In Irritable Bowel Syndrome

REFERENCES **223**

CHAPTER ONE, SECTION 1:

CLINICAL PRESENTATION OF IRRITABLE BOWEL SYNDROME

EPIDEMIOLOGY AND SOCIAL IMPACT OF IRRITABLE BOWEL SYNDROME

Irritable Bowel Syndrome (IBS) is a functional gastroenterological disorder (FGID) whose symptom complex comprises recurrent abdominal pain or discomfort with disordered bowel habit. Symptoms may be chronic and relapsing with only a minority of patients enjoying a return to a normal pre-morbid state.[1] Diagnosis has traditionally been secured by the exclusion of other viable organic pathology, such as inflammatory bowel disease or coeliac disease, in the presence of symptomatology consistent with the syndrome. As such no specific test or diagnostic process for IBS exists. Therefore a physician is presented with a dilemma at the point of diagnosis because he has to make a clinical judgement about whether a patient with a typical presentation should be subjected to the full gamut of clinical investigation, for example colonoscopy, with all the concomitant risks and associated costs of that process. From the perspective of the patient, the combination of invasive testing with the subsequent negative findings and the lack of any cohesive system of patient stratification is equally frustrating.[2]

EPIDEMIOLOGY

Prevalence

Irritable Bowel Syndrome is a common condition with 10 to 20% prevalence in the United Kingdom.[3] However the true prevalence of IBS may be masked by a population of patients who do not seek health-care services.

Age

Age distribution sees peak prevalence during the 20 to 30 year old age range. Prevalence of IBS decreases with age.[4] However with increasing awareness of the syndrome, a discrete yet significant elderly population has been identified.[5,6] Differential diagnoses must be excluded in this age range before a diagnosis can be made. This is due to increasing medical co-morbidity and polypharmacy with increasing age. A community-based survey performed by Talley in 1995 reported that 23% of IBS patients over 60 years of age had initial symptom onset within 12 months compared to just 10% of those of a younger age. Again, true prevalence in the elderly population cannot be reliably ascertained, since elderly patients are less likely than younger patients to seek health care services.[7]

Incidence

Up to 10% of the population will have symptoms consistent with IBS during a 12-month period. 5% of the population will seek primary health care services during this period of time.

Gender

Most population-based studies report that females are more commonly affected than men by a factor of between two and three times to one.[4] Some studies report an equal sex distribution.[8] Composite scores for symptom severity and infringements on activities of daily living were studied with regard to gender and medical point of entry in a study by van der Horst.[9] This showed that symptom severity between the sexes was similar in outpatient gastroenterology clinics, but that women felt that their symptoms were more serious and interfering than their male counterparts in the primary care setting. With abdominal pain as the prominent IBS symptom, there seems to be an equal distribution between sexes. However females are more predisposed to constipation, and males are more predisposed to diarrhoea.[10] Corney concluded that women were more severely affected by physical symptoms with the exception of diarrhoea, and were more likely to be in pain longer and for more days in the month.[11] Differentiation between the sexes has also been noticed with regard to extra-gastrointestinal symptoms. Lee reported a constellation of extra-colonic symptoms that were more common in females.[12] These symptoms included abdominal distension, bloating, nausea, alterations of taste and smell, unpleasant sensations on the tongue, muscle stiffness in the morning, greater food sensitivity and side effects from medications.

Symptom severity is influenced by the menstrual cycle. IBS patients were compared to controls by studying somatic and psychological symptom severity during the follicular and luteal phases of the menstrual cycle[13]: IBS patients had higher severity scores regardless of oral contraceptive use or predominant bowel pattern, with an amplification of pain severity noticed during the late luteal and early menses phases of the cycle.

Whitehead approached this subject from a different angle- he identified 383 females with a diagnosis of dysmenorrhoea, and reported that 61% had symptoms consistent with a diagnosis of functional bowel disorder, defined as abdominal pain with altered bowel function.[14] Houghton assessed rectal sensitivity using recto-sigmoid distension during the menstrual cycle.[15] This study documented increased rectal sensitivity in IBS patients during the menses, whereas rectal sensitivity remained unchanged throughout the menstrual cycle in the control population. Houghton surmised that female IBS patients responded differently to fluctuations in the sex hormonal environment compared to controls.

Culture

The lack of a reliable diagnostic marker for IBS has made the task of constructing reliable epidemiological cross-cultural studies extremely difficult. The methodology used for identifying cases in such studies has significant variability. Diagnostic criteria has evolved over the course of many years from being non-specific to the adoption of the Manning criteria, and the later incorporation of the Rome criteria, which is itself under continuous refinement and evolution. Assessment of prevalence across different cultures can only be applicable if the methodology of the studies under analysis is at least broadly similar. Furthermore, most of the epidemiological studies have concentrated on the white Western population. Ethnic comparisons may be confounded by factors such as a lack of uniformity of sampling sizes, composition of the study populations and research methodology. A further consideration is whether to study ethnic populations in their native or non-native environments.

Western populations appear to have higher rates of prevalence of IBS than non-Western populations. This may reflect a true disparity in prevalence, either through cultural or biological mechanisms, or may be secondary to intrinsic deficiencies of the research protocols and the difficulties in assessing the target populations. There is now an impetus for the delivery of reliable and robust cross-cultural research competencies. Much work has been done recently in assessing the validity of the Rome 3 criteria in different cultural contexts.

The relationship between culture and illness perception defines the subjective impact of the illness on the individual. Illness beliefs, illness behaviour, the adoption of the sick role and psychological coping mechanisms are influenced by one's culture. This is in addition to any cultural physiological differences that may be present. Cross-cultural differences in epidemiological studies may also reflect differences in socio-economic status, literacy, schooling, political affiliation and healthcare provision. Wigington demonstrated lower prevalence of IBS in African Americans compared to white Americans (RR=-2.5), but both groups shared the same quality of life disease burden.[16] Subsequent research by the same group questioned whether race affected treatment outcome. They found that Caucasian Americans with IBS were far more likely to be proposed for surgery (appendicectomy) for flares of abdominal pain than African Americans (21.4% vs. 1.9% ($P < 0.01$).[17] This invites discussion on the relationship between culture, the diagnostic process and health care allocation.[18,19]

SOCIAL IMPACT

Quality Of Life

Quality of life studies show that the psychosocial impact of IBS is considerable.[20,21] A study by Jones compared quality of life scores between IBS patients, inflammatory bowel disease (IBD) patients and control subjects. The results showed that impairment in quality of life was as significant in the IBS group as the IBD group. Gralnek demonstrated that IBS patients scored significantly lower on all of the SF-36 Health Survey scales ($P < 0.001$) except physical functioning. Similarly, IBS patients had significantly worse health-related quality of life scores on selected SF-36 scales than patients with diabetes mellitus and end stage renal disease.[22] However, it is important to note that quality of life impairment is not entirely attributable to somatic or gastrointestinal symptomatology.

Recent studies suggest that the concomitant burden of psychosocial factors has a significant negative impact on quality of life. Creed enrolled a cohort of 257 patients with refractory IBS to assess the relationship between quality of life, physical symptoms and psychological factors.[23] The results show that abdominal and psychological symptoms were independently associated with impaired health-related quality of life in patients with severe IBS. An interesting study by Simrén sought to identify predictive factors for impaired quality of life in IBS patients.[24] He found that extra-colonic factors were the main determinants of impaired quality of life. These included factors such as easy fatigueability, anergia, sexual dysfunction and feeling tense or nervous. As a consequence, he proposed that physicians should turn their attention towards addressing patients' global symptom severity and anxiety levels rather than obsessing with the vagaries of bowel dysfunction and stool consistency.

Absenteeism

A large cross-cultural population-based study with 41,894 individuals in 8 different countries noted that IBS patients reported having more sickness days off work in the previous 12 months than the control population.[25] They spent 3.9 days in bed (compared to 2.7 days in the controls), 5.5 days off work through illness (compared to 3.1 days in the controls), 10.2 days when work activities had to be cut short (compared to 4.8 days in the controls) and 8.4 days consulting a doctor or nurse (compared to 5.2 days in the controls). Of those who had formally diagnosed irritable bowel syndrome only 10% had informed their employer of their condition.

A study by Akehurst et al. published that 45% of their population of IBS patients had taken time off work with symptoms related to their condition. Of these, 15% had taken more than 1 week off work in the previous 3 months prior to entry into the study.[21]

Health-seeking Behaviour

IBS is the most likely reason why a patient will present to a gastroenterological clinic. The majority of IBS patients do not seek health care services.[3] Estimates suggest that up to 3.9 million people seek medical consultation in England and Wales during a 12 month period. Women are more likely to seek health care services than men.[26] With reference to the fact that functional gastrointestinal disorders (FGIDs) are so common, the decision to seek health care may introduce bias into research.[27]

Surgery

Irritable Bowel Syndrome is associated with an increased risk of surgery even if major confounding variables such as age have been removed. A study published by Longstreth et al. examined a dataset of 89,000 patients to investigate the propensity of IBS patients to undergo surgery.[28] Several confounding variables were corrected for, yet despite this, IBS patients were two times more likely to have an appendicectomy or hysterectomy, three times more likely to have a cholecystectomy and 50% more likely to have back surgery compared to non-IBS controls. Data collected from a UK sample showed that IBS patients were almost twice as likely to have cholecystectomies.[29]

Suicide

Feelings of hopelessness, the severity of one's symptoms, and the ineffectiveness of treatment in Irritable Bowel Syndrome can collude with fatal consequences. Peter Whorwell's group sought to compare suicidal ideation secondary to concurrent gastrointestinal symptomatology in IBS patients in primary, secondary and tertiary care settings and to use an IBD population as a positive control.[30] The data showed that 38% of the tertiary care IBS patients had contemplated suicide because of their symptoms compared with 16% and 4% of the secondary and primary care IBS patients. In comparison, 15% of the IBD patients acknowledged thoughts of suicide. Of the 400 patients studied (100 patients per group), 5 tertiary setting IBS patients and 1 IBD patient had previously attempted suicide. Increasing scores for depression had a positive correlation with suicidal ideation. However depression, anxiety and symptom severity could not account for all the observed differences.

Spiegel's meta-analysis was constructed to remove the variable of psychiatric comorbidity from those patients with IBS (or recurrent abdominal pain) who expressed suicidal ideation.[31] IBS patients were two to four times more likely to have suicidal behaviour compared to controls. After adjustments for psychological comorbidity were made, chronic abdominal pain was an independent predictor of suicidal behaviour.

Direct Costs of Irritable Bowel Syndrome

Maxion-Bergemann et al. performed a systematic literature review on 18 original papers from the UK and US that assessed the direct and indirect costs of Irritable Bowel Syndrome.[32] Total direct cost estimates per patient per year ranged from US $348 to $8750 whilst indirect costs ranged from US $355 dollars to US 3344 dollars. Due to the different methodologies used in the individual papers, comparison between US and UK costings proved impossible. However, the study concluded that IBS patients had an increased economic burden of healthcare costs that was between 1.1 and 6.0 times that of matched non-IBS controls. An estimate of the total cost of IBS health care provision in the US was $1.58 billion in 1998.[33]

Indirect Costs of Irritable Bowel Syndrome

Bentkover et al. analysed the indirect costs of Irritable Bowel Syndrome to the Canadian economy.[34] An estimated annual figure of 748 Canadian dollars was calculated by multiplying the time lost at work with the mean Canadian wage. Similar calculations were performed by Creed et al. who determined that the annual cost to the American taxpayer was $335 per IBS patient.[23] Sandler's review assessed indirect costs as being productivity losses caused by absence from paid employment resulting from the consumption of health care.[35] It did not try to calculate other more intangible costs such as diminished productivity in the workplace, absence from work, not receiving medical care or the loss of future earnings due to disease severity. This study estimated that the cost to the American health system was $205 million annually (taken from data in 1998).

DIAGNOSIS OF IRRITABLE BOWEL SYNDROME

CLINICAL PRESENTATION

The Rome Committee

Irritable Bowel Syndrome is defined by the Rome criteria. The Rome criteria have been developed over the years by a collective of international gastroenterologists who meet periodically to define and then redefine the functional gastrointestinal disorders (FGIDs). The Rome committee was founded in 1989 following on from initiatives by Professor Aldo Torsoli who invited investigators to contribute to a consensus-led approach to the development of diagnostic criteria for FGIDs. This process saw its birth at the International Congress of Gastroenterology in Rome in 1988- this is where the committee acquired its name and direction. Professor Torsoli encouraged the implementation of a Delphi method of research communion. This is a process that favours consensus-led research by seeking unanimity and consistency of opinion from a panel of experts rather than the individual. The infrastructure of the Rome Committee now demands a rigorous four year 13 step cycle of evolution. The last Rome convention was in 2006, in which they published the Rome 3 criteria for IBS.[36] Ongoing research will see the publishing of the Rome 4 criteria in approximately 2014.

Manning Criteria

Manning et al. published their landmark paper in 1978 entitled “Towards positive diagnosis of the irritable bowel”. They assessed a prospective cohort of 109 unselected and undiagnosed patients who had been referred for specialist assessment of the symptoms of abdominal pain, constipation and diarrhoea. A 15 point questionnaire had been devised to enquire about the various subtleties of bowel dysfunction such as nocturnal bowel movements, mucus per rectum or pain easing after bowel movement. The patients were reviewed between 17 and 26 months later by two independent gastroenterologists who had been blinded to the results of the initial questionnaire. During the intermission between questionnaire and later clinical review, the patients had been subjected to full comprehensive investigation to establish each individual diagnosis. The questionnaire items were compared to the underlying diagnosis, be it a functional or an organic cause. They found that of the 15 questions, 4 questions clearly discriminated between functional and organic disease. The questions favouring a functional diagnosis asked whether a patient became visibly distended, had relief of pain with defaecation, or more frequent or loose stools with the onset of pain. The Manning criteria laid the foundation for the initial Rome criteria, and the Rome Foundation that ensued.

Manning Questionnaire

The patient should present with at least 2 of the following symptoms for an IBS diagnosis to be made:

- Onset of pain associated with more frequent bowel movements
- Onset of pain associated with more loose bowel movements
- Relief of pain with defaecation
- Abdominal distension
- Sensation of incomplete evacuation with defaecation
- Passage of mucus

Kruis Criteria

Kruis combined elements of the clinical history, examination and basic investigation to create a scoring system for the diagnosis of IBS.[37] The study also undertook to qualify the sensitivity and specificity of the proposed scoring system, thus allowing the calculation of positive predictive values.

479 unselected outpatients referred to a gastrointestinal clinic were examined clinically and by investigation with upper and lower gastrointestinal endoscopy, abdominal ultrasound and routine blood tests. Of the 479 subjects, 108 were diagnosed with IBS and 209 were diagnosed with organic disease. A scoring system was devised. This incorporated a patient questionnaire and the results of select clinical parameters and investigations. Logistic regression analysis computed a weighted scoring system. Using the weighted score the specificity was 99% with a sensitivity of 67% for the diagnosis of irritable bowel syndrome. It was thought that the scoring system was sufficiently powered to discriminate IBS from organic pathologies.

Kruis Patient Questionnaire

- Did you come because of abdominal pain? YES / NO
- Do you suffer from flatulence? YES / NO
- Do you suffer irregular bowel movements? YES / NO
- Have you experienced this for > 2 years? YES / NO
- How can your abdominal pain be described: burning, cutting, very strong, terrible, feeling of pressure, dull, boring, not so bad? YES / NO
- Have you alternating diarrhoea/constipation? YES / NO

- Have your stools any of the following properties? pencil-like; rabbit pellets; hard in the first portion and looser in the second portion; mucus? YES / NO

If the patient answers yes in any of sections of each question, a scoring system is allocated as follows:

- Question 1: 34 points
- Question 2: 16 points
- Question 3: 23 points
- Question 4: 14 points
- Question 5: No score

Maximum score: 87 points

The patient questionnaire is then validated by the clinician- he or she identifies markers or indicators of disease and potential red flags, and then makes appropriate subtractions from the original total.

Kruis Clinician Questionnaire

- Abnormal physical findings, and/or history for alternative diagnosis YES / NO
- ESR > 20 YES / NO
- Leucocytosis > 10,000 YES / NO
- Haemoglobin: Females < 12 g/dl, Males < 14 g/dl YES / NO
- History of blood in stool YES / NO
- Fever (> 38.5) in the last week YES / NO
- Underweight YES / NO
- Loss of weight > 5 kg in last 6 months YES / NO

If the clinician answers yes to questions 1 – 5, a scoring system is allocated as follows:

- Question 1: 47 points
- Question 2: 13 points
- Question 3: 50 points
- Question 4: 98 points
- Question 5: 98 points
- Questions 6–8: No score

This is then subtracted from the original patient score. Scores greater than 44 indicate positive IBS cases.

Rome Criteria

The Rome criteria were published in 1990 following the proposals of the International Conference of Gastroenterology in 1998. It defined the symptom criteria required to make a diagnosis of IBS. It was informed by the original Manning criteria[38] and the subsequent Kruis criteria.[37]

Rome 1

The Rome Committee began in earnest in 1990 with the task of characterising the FGIDs including IBS. They identified 21 distinct conditions that required symptom-based definition. Owing to limited systems of promotion, the Rome process was still a relatively unknown entity to both gastroenterologists and general physicians alike. As a result there was little penetration of the Rome criteria into the consciousness of the physician and investigator. The Rome 1 criteria was significant in that it introduced abdominal pain as a central symptom for IBS- the original Rome criteria had not made this distinction. The Rome 1 criteria were published in 1992.

Rome 1 Criteria

The patient should present with at least 3 months of continuous or recurrent symptoms for an IBS diagnosis to be made:

Abdominal pain or discomfort, which is:

- Relieved with defaecation
- and/or associated with altered bowel frequency
- and/or associated with altered stool consistency

and/or two or more of the following, on at least 1/4 of days:

- Altered stool frequency
- Altered stool form
- Altered stool passage (straining, urgency or tenesmus)
- Passage of mucus

Other supporting symptoms include bloating or a feeling of abdominal distension.

Rome 2

The Rome 2 criteria were published in 2000. Its development was encouraged by increasing interest from physicians and the pharmaceuticals industry. Significant funding streams were secured from industry to support the Rome process. This allowed for the expansion of the number of committee groups that assessed specific classes of FGID and enabled a more international contribution of research consensus.

The Rome 2 criteria define IBS as recurrent abdominal pain or discomfort that presents over a 12 week period in the preceding 12 months. The symptomatic weeks do not have to be consecutive. These cardinal features of IBS may be accompanied by other supporting symptoms, such as bloating, lethargy, urinary frequency, backache and dyspepsia.

The Rome committee acknowledged considerable overlap between various subtypes of FGID. As an example, IBS patients have a similar clinical presentation to those with functional constipation (in whom the infrequency of defaecation leads to abdominal distension and discomfort).

Rome 2 Criteria

Twelve inconsecutive weeks or more in the past 12 months of abdominal discomfort or pain that has 2 out of 3 features:

- Relief with defaecation
- Associated with a change in the frequency of defaecation
- Associated with a change in the consistency of the stool

Associated symptoms which would give confidence to the clinician that pain was of an abdominal origin:

- Abnormal stool frequency (> 3/day or < 3/week)
- Abnormal stool form (lumpy/hard or loose/watery) > 1/4 of bowel motions
- Abnormal stool passage (straining, urgency or feeling of incomplete evacuation) > 1/4 of bowel motions
- Passage of mucus > 1/4 of bowel motions
- Bloating or feeling of abdominal distension > 1/4 of days

Rome 3

The Rome 3 criteria were published in 2007 encompassing the committee's recommendations in light of advances in the medical literature. The most significant change was the timing of symptoms. The Rome 2 criteria stipulated the requirement of symptoms to be present for 12 non-consecutive

weeks in a 12 month period. The Rome 3 criteria now advocated that symptoms should be present for 3 months in a 6 month period. The committee felt that the new time frame was less restrictive, and easier to apply in the clinical and research environment.

Another modification to the definition of IBS was that the sub-typing of IBS patients into diarrhoea-predominant, constipation-predominant or mixed-type groups should be based on stool consistency alone (rather than any reference to frequency of defaecation).

With reference to the other FGIDs, the Rome Foundation significantly expanded their classification of adolescent and paediatric disorders.

Rome 3 Criteria

Symptoms of recurrent abdominal pain or discomfort and a marked change in bowel habit for at least six months, with symptoms experienced on at least three days of at least three months.

Two or more of the following must apply:

- Pain is relieved by a bowel movement
- Onset of pain is related to a change in frequency of stool
- Onset of pain is related to a change in the appearance of stool

The Rome committee recommended that for IBS patients to be included into clinical trials, they should experience abdominal pain or discomfort for at least two days of the week.

Rome 1 vs. Rome 2 vs. Rome 3 vs. Manning Criteria: Implications for Research

With the constant evolution of the diagnostic criteria for Irritable Bowel Syndrome, there is a considerable variation in the prevalence of the condition according to which criteria is employed. Mearin et al. compared the Manning, Rome 1 and Rome 2 criteria in a population-based survey of 2000 randomised Spanish individuals, matched according to age, gender, socio-economic class and location.[39] Prevalence of IBS was 10.3% (Manning criteria), 12.1% (Rome 1 criteria) and 3.3% (Rome 2). Indeed, more than two-thirds of the subjects who fulfilled the Rome I criteria would have not have been diagnosed with IBS if the Rome 2 criteria had been used. This led Boyce et al. to question whether the Rome 2 criteria was unduly restrictive in clinical practice.[40]

The Rome 3 criteria are less limited in terms of duration of symptoms, and calculates a higher prevalence of positive IBS cases. A study by Sperber et al. documented an extremely low prevalence of IBS patients in a population of adult Israeli Jews using the Rome 2 criteria.[41] Using that same population sample, the Rome 3 criteria were retrospectively applied in a later study.[41] The

prevalence of IBS rose dramatically from the original study figure of 2.9% to 11.4% when applying the Rome 3 criteria.

The selection of diagnostic criteria has important ramifications in terms of identifying cases, patient management and the construction of research methodology.

Approaching a Clinical Diagnosis

A diagnosis of IBS can only be made when there has been adequate provision to exclude other disease processes.[42] A robust clinical history will identify any red flag symptoms that might point to an organic pathology. These include unintentional and unexplained weight loss, rectal bleeding, and a family history of bowel or ovarian cancer.

Examination of the patient may suggest clues pointing towards organic pathology- clinical signs such as cachexia, organomegaly or rectal masses would certainly warrant further investigation. Recent interest has centred on the early presentation of ovarian cancer mimicking IBS. If an ovarian cause were suspected, a pelvic examination with pelvic and/or trans-vaginal ultrasound and cancer antigen testing (CA-125) would be required.

Red Flag Symptoms

The addition of red flag symptoms to the Manning criteria improves diagnostic accuracy. In a retrospective analysis of 98 patients, Vanner et al. combined the absence of red flag symptoms with the Rome 1 criteria, reporting a sensitivity of 65%, specificity of 100%, and positive predictive value of 100%.[43] The same study also prospectively assessed 95 patients who were Rome 1 positive for IBS and red flag symptom negative. The positive predictive value in this group was 98%- none of these patients required revision of their diagnosis during a 2 year follow-up.

Alarm Symptoms

History

- Age of onset ≥ 50 years old
- Weight loss
- Nocturnal or refractory diarrhoea
- Significant travel history suggestive of GI infection
- Severe constipation or diarrhoea
- Rectal bleeding
- Rashes/arthritis

Family History

- Coeliac disease
- Colon cancer/polyps
- Inflammatory bowel disease

Alarm Signs

- Physical Examination
- Oral ulcers
- Fever
- Abdominal or rectal mass
- Rectal bleeding
- Rash

General Approach

A vital goal is to establish a constructive doctor/patient relationship. Research has proven that this will improve patient satisfaction, symptom improvement and compliance of therapy.[44,45] Drossman et al. published a set of guidelines to aid this process.

These guidelines are summarised:

1. Use a non-judgmental patient-centred approach to taking the history.
2. Assess what the patient's level of understanding of their condition.
3. Be realistic in responding to a patient's expectations.
4. Assess what exacerbating factors are present, with particular regard to stressors.
5. Establish consistent limits, for example with regard to analgesic use.
6. Examine the patient and implement cost-effective investigation.
7. Involve the patient in the decision-making process.
8. Pursue the best interests of the patient.
9. Establish robust links with the primary care giver of each and every patient.

The above approach should establish a robust rapport with a patient. However, it is also important to identify specific exacerbating factors that have brought the patient into the clinic on that particular day. These factors include whether the patient can identify specific exacerbating factors, what the concerns of the patient are, whether there are any environmental factors or psychological comorbidities, and whether there are any disturbances in daily functioning. Finally, it is important to identify more worrying underlying motivations, such as narcotic abuse, disability allowances etc.

The clinician needs to be able to discern the physiological and psychological mechanisms that are driving a patient's symptoms, address psychosocial issues and direct treatment accordingly. Treatment of IBS will be determined by an assessment of clinical severity.

SYMPTOM VARIABILITY

IBS Sub-types

Patients with Irritable Bowel Syndrome have traditionally been categorised according to the prevailing bowel habit, for example a constipation-predominant phenotype. The grouping of patients into clinical sub-types seems to imply to the non-specialist that patients within each group have a shared aetiology to their symptoms. Unfortunately this is not the case. Furthermore there is ample opportunity for patients to flit between various sub-types over a period of time.[46]

IBS patients may fall into diarrhoea-predominant (IBS-D), constipation-predominant (IBS-C), mixed (IBS-M) or unsubtyped (IBS-U) subtypes. The Rome 2 criteria demanded that in addition to an assessment of the consistency of the stool, the prevailing bowel subtype should be qualified by time- abnormal frequency of defaecation was defined as opening the bowels less than once every 3 days or more than 3 times in a single day.

Stool consistency is qualified by the ubiquitous Bristol Stool chart. This is a graphical representation of stool consistency, graded from Type 1 (small, hard pellets of stool) though to Type 7 (entirely liquid stool)

Garrigues et al. assessed 517 IBS patients over a 12 month period using diary cards and the Bristol stool chart.[47] The bowel habit remained unchanged in a third of the patients, and the frequency of constipation and diarrhoea showed few changes during the period of follow-up. Approximately 50% of the changes in bowel habit occurred from/to IBS-M and IBS-U, and 14% of the changes occurred from IBS-C to IBS-D or vice versa. This last example reveals the IBS alternator, a subtype significantly associated with the female gender (OR= 2.65). Drossman recommended that the IBS alternator (IBS-A) be defined as at least one change between IBS-D and IBS-C by Rome II criteria over a 1-year period.[46]

Natural history

The natural history of Irritable Bowel Syndrome is hugely variable, though most investigators and patients would agree that the syndrome is a life-long affliction and that symptoms and symptom severity may vary over time.

A systematic review by El-Serag et al. determined that 2-5% of patients who completed baseline investigations were misdiagnosed as having IBS when in fact organic disease was present (follow-up time was 6 months to 6 years), though this range increases to 2-9% if 30 year follow-up data is included. One paper under review reported that 88% of patients had chronic symptoms at 12

months follow-up, and that there was an 81% concordance of symptoms in the same patients (who were still symptomatic) after a further 31 month follow-up.[48] Four studies reported on the proportion of patients with unchanged symptoms (30–50%) and patients whose symptoms had deteriorated (2–18%). Finally, 5 of the 14 papers calculated that the proportion of patients with complete disappearance of symptoms ranged from 12 to 38% (median follow-up duration was 2 years).

An interesting study by Talley conducted a postal symptom questionnaire on a random population of residents in Olmsted Co, Minnesota.[49] The questionnaire was resubmitted to the same population over a year later to assess symptom reproducibility. After correction for age and gender, the prevalence rates for IBS were similar at the two time points. However, 9% of the individuals who were initially symptom-free had acquired symptoms consistent with a diagnosis of IBS, and 38% of those with a diagnosis of IBS at baseline had become asymptomatic by the second questionnaire.

Lembo et al. assessed symptom duration, rectal sensitivity and psychometric studies between controls, IBS patients with recent symptom onset and IBS patients with a long history of the condition.[50] After the initial assessment, all subjects were invited to reappraise their symptoms with a follow-up questionnaire. The data reported that IBS patients with a recent onset of symptoms and fewer psychological symptoms had a better prognosis than patients with long-standing disease and significant psychological comorbidity.

Associated Functional Gastrointestinal Disorders

Overlap of symptoms between any two FGIDs can range between 4% and 9% of the population.[51] This can create diagnostic uncertainty, confusion for the patient, and questions over optimal treatment approach. Furthermore, the presence of overlapping FGIDs suggests a common pathophysiology which is dissonant to the accepted Rome understanding that FGIDs are discrete entities.

Agréus et al. compared IBS with dyspepsia in a general population over 12 months.[52] Casual analysis of their data shows that prevalence of the two conditions remained stable over the course of the study. However closer inspection revealed that 50% of patients (from either group) changed their symptom profile and that 87% of IBS patients fulfilled the criteria for dyspepsia. They concluded that much of the variability in terms of onset and resolution of each disorder was due to the fact that symptoms were being attributed to a different functional disorder rather than actual symptom

resolution. This conclusion was endorsed by Talley's group which published a 12 year longitudinal population-based study that assessed FGIDs.[53] Data from this study reported that of those symptomatic patients at baseline approximately 20% had the same symptoms, 40% had no symptoms, and 40% had different symptoms at follow-up, more than a decade later.

ASSOCIATED NON-GASTROINTESTINAL NON-PSYCHIATRIC SYMPTOMATOLOGY

Non-gastrointestinal non-psychiatric symptoms are common in Irritable Bowel Syndrome. Whorwell assessed 100 patients with IBS by documenting extra-colonic features.[54] Symptoms included nocturia, frequency and urgency of micturition, incomplete bladder emptying, back pain, an unpleasant taste in the mouth, a constant feeling of tiredness and dyspareunia. The diversity of extra-colonic symptoms remained even when concomitant psychiatric comorbidity had been corrected for. This highlights how Irritable Bowel Syndrome can present with a multiplicity of symptoms external to the bowel- a competent physician will be able to acknowledge these complimentary symptoms, and not progress down an avenue of inappropriate investigation or referral.

In terms of non-gastrointestinal non-psychiatric comorbidity, Whitehead published that temporomandibular joint disorder (TMJD)[55] was the most common disorder associated with IBS (a median of 64% of patients with TMJD have IBS). Other associations include fibromyalgia (median of 49% have IBS), chronic fatigue syndrome (51%) and chronic pelvic pain (50%). A reasonable conclusion from this is that there is a common pathogenetic pathway, perhaps suggesting an underlying somatoform disorder. However, multivariate analysis of the dataset recommended that the each disease state was a discrete entity. An alternate explanation is that if multiple disorders are present then a primary psychogenic mechanism may be present.

PSYCHIATRIC COMORBIDITY

Irritable Bowel Syndrome is not associated with any one psychiatric disorder. However, when symptoms of anxiety or depression become prominent and persistent, a formal psychiatric diagnosis may be considered. Indeed, the recognition and assessment of concomitant psychiatric disorders must be given as much attention as the treatment of the gastrointestinal symptomatology.

Studies show that the prevalence of psychiatric comorbidity is high- affective or anxiety disorders have a prevalence of between 42% and 61% in FGIDs. This contrasts with a prevalence of the same

conditions of between 6 and 25% in patients with established organic gastrointestinal disease, and 8 to 18% in control subjects.[11,56-60]

The association between psychiatric comorbidity and Irritable Bowel Syndrome is heightened in IBS patients who seek medical services.[61] Sandler et al. observed that patients with bowel dysfunction who presented for medical consultation were more likely to have abdominal pain than the non-consulters, but this could not fully explain the reason for the visits to the doctor.[62] In addition, those patients going to the doctor were more likely to be concerned about non-gastrointestinal symptoms. Given the accepted prevalence of psychological comorbidity in IBS, this could suggest altered behavioural influences that precipitate health care seeking.

Kettell reviewed 24 IBS consulters and 24 IBS non-consulters with an array of psychometric tests.[63] The striking difference between the two groups was that mean negative life event scores, and scores for anxiety and depression were higher in the group who consulted. The consulting patients seemed to be more concerned about the potentially serious nature of their symptoms, with 10 of the 24 consulting patients speculating that they may have cancer.

Life events

Serious life events may serve as the precipitant for the onset of FGIDs, or may act to propagate symptomatology. The strength of this association is marked- Creed et al. observed that two thirds of patients with FGIDs acknowledged a serious life event compared to a quarter of the control population under review.[64]

Whitehead corrected for concomitant psychiatric disease yet still found that there was significantly higher levels of stress in an IBS cohort compared to controls or patients with non-IBS functional disorders.[65] Even more interesting was the analysis of the time course of stressful events- the introduction of a stressor in a three month period was significantly associated with the development or worsening of bowel dysfunction in the following three month period. Stress was positively correlated with the number of disability days (days off work or days seeking medical services). Regression analysis showed that the regression line relating stress to bowel symptoms was steeper in IBS than in controls and (non-IBS) functional bowel disease. This suggests that IBS patients are hypersensitive to stressful environments.

Chronic life stress measures and symptom severity markers were performed on IBS patients over a 16 month longitudinal study period.[66] The results concluded that chronic life stress was a powerful predictor of symptom intensity, and that nearly all of the variance of an individual's symptom intensity could be explained by the severity of chronic threat during the previous 6 months or more. Furthermore, for the majority of IBS patients, stress above a critical threshold predicts a failure of symptom improvement or resolution.

Abuse

The association of Irritable Bowel Syndrome with previous physical or sexual abuse is a controversial and inflammatory subject. The link was first proposed by Drossman in 1990.[67] He assessed a consecutive cohort of female patients with functional symptoms who had been referred to a university-based gastroenterology practice. A staggering 44% of the 208 patients admitted to a history of sexual or physical abuse. Nearly all of those who had been physically abused were sexually abused. Furthermore, approximately a third of patients had never admitted this fact before, and only 17% confided that their gastroenterologists were aware of the abuse. By comparing this group to a population with organic disease, functional patients were more likely to report forced intercourse, physical abuse, chronic abdominal pain and increased surgical procedures. This study not only suggested a causative link, but more disturbingly it highlighted that abuse was prevalent, concealed and recurrent.

Supporting data came from Talley's group.[68] This study extended the line of enquiry by questioning whether abuse was prevalent in community-based IBS patients, as opposed to those who had been referred on to secondary or tertiary centres. 919 randomised individuals in the community responded to a self-report postal questionnaire. Age and sex-adjusted prevalence of abuse was 26%, and there was a significant association between IBS and sexual abuse, emotional or verbal abuse, and abuse in childhood and adulthood. Furthermore, those presenting to gastroenterologists for specialist opinion were more likely to have had an abuse history, suggesting increased health care utilisation in this group of patients.

A study a year later by the same group compared an outpatient sample of functional patients with patients with organic disease.[69] Differences in abuse prevalence were not significant between the two groups. However, IBS-type symptoms were more prominent in those patients who reported abuse than those who did not (OR= 1.7).

Leroi et al. evaluated the relationship between sexual abuse and distribution of symptoms.[70] 344 patients presenting to either a university-based tertiary referral centre or a gastroenterology private practice were categorised according to whether they had a functional or an organic presentation. 40% of the functional patients admitted sexual abuse compared to 10% of the organic patients. Analysis of symptom distribution revealed that abused patients were more likely to complain of constipation and diarrhoea. Anismus was more common in sexual abuse, and the prevalence of abuse was significantly greater in patients with lower rather than more proximal functional gastrointestinal symptoms ($P < 0.002$).

Mood and Anxiety Disorders

Associated mood disorders in IBS include depression and dysthymic states. Anxiety disorders include panic, generalised anxiety and post-traumatic stress disorder. The severity of psychological distress is closely associated with health care-seeking behaviour. Those IBS patients who present with psychological distress are more amenable to psychological intervention than patients who have personality disorders.

Gwee et al. utilised psychometric testing in post-gastroenteritis patients over a 6 month period.[71] Of the patients who went on to be diagnosed with IBS, increased baseline scores were seen for anxiety, depression, somatisation, and neurotic traits. Repeat assessment of these psychological parameters 3 months after the initial illness showed that the psychological distress was maintained over time.

Spiller's group extended the scope of Gwee's earlier work by constructing a study incorporating a 6 year follow-up of post-gastroenteritis patients.[72] This study demonstrated that anxiety and depression can be important predictors in the development and maintenance of symptoms in post-infectious IBS- only one patient with a positive history of anxiety or depression had symptom resolution after the initial illness. This was one subject out of a total of 24 patients who had been diagnosed with IBS from the original cohort of 436 post-GE patients.

Personality disorders

Research has failed to identify a psychological trait that is common to IBS patients. Controlled studies have shown that anxiety and neurotic traits may be raised in IBS consulters compared to IBS non-consulters or controls[61] However, such deductions may be confounded by the self-selection of IBS patients presenting to health care services.[73] Other potential confounders when investigating

personality disorders in IBS are the fact that IBS patients may score more highly on personality scores when symptomatic, and that personality scores will reduce on symptom resolution.[74] This makes inferences about causality difficult.

The anxious or avoidant personality disorder may underlie increased anxiety levels seen in a limited subset of IBS patients. Personality disorders are deeply ingrained traits that present during early adult life, and are characterised by rigid, persistent patterns of cognition, emotions and behaviour. The underlying belief systems informing these patterns of behaviour are referred to as fixed fantasies or dysfunctional schemata. The inflexibility and pervasiveness of behavioural patterns arising from a personality disorder may precipitate significant social dysfunction in an individual. Furthermore, treatment of a personality disorder is notoriously difficult to treat.

Talley explored the link between abuse, neuroticism and Irritable Bowel Syndrome.[75] Univariate analysis showed that IBS was correlated with childhood but not adult abuse, and that neuroticism and psychological morbidity were associated with abuse in childhood, abuse in adulthood, and IBS. After controlling for age, gender and psychological factors, the relationship between childhood abuse and IBS was not maintained. This prompted speculation that childhood abuse may stimulate the expression of neuroticism which can then precipitate the later development of IBS.

DIFFERENTIAL DIAGNOSIS

Organic processes which mimic the symptoms of IBS are legion. Differential diagnoses range from inflammatory bowel disease, gynaecological disease, the causes of malabsorption (for example Coeliac disease), neoplasia, infection, endocrine dysfunction, psychiatric disease and food intolerance. Such a broad range of differential diagnoses presents a diagnostic challenge to the physician and an economic challenge to the government. With a 10 to 20% UK prevalence, and a 50% likelihood that a patient with symptoms will present to primary care services, the cost implications are enormous.[32] Emerging trends highlight the prevalence of newly diagnosed IBS in the elderly population.[6,76] This is an age group which is characterised by polypharmacy and co-morbidity, further confusing the diagnostic process. Therefore recent interest has centred on the importance of finding the right test for the right patient.

Food Allergy and Intolerance

Adverse reactions to food may result from direct toxicity of the ingested food or an immune-response to non-toxic foods. Immune-reactions to food are denoted food allergies, and may involve IgE pathways.[77] In contrast, food intolerance is a non-immune-mediated response to immunological, pharmacological, toxic, infectious, idiosyncratic, metabolic and neuropsychological processes.[78] Many people in the community feel that they have food intolerance, as evidenced by a high response rate in self-reporting questionnaires. Burr et al. reported food intolerance in 19% of men and 26% of women who responded to their self-report questionnaires. Of the 524 subjects polled, a random subset of 99 females had blood tests to quantify plasma IgE levels. The plasma IgE levels were found to be significantly *lower* in this group, suggesting that food allergy was not a mechanism driving their food intolerance.[79]

In summary, dietary exclusion may be of value to a minority of IBS patients, but the diagnosis of food intolerance should be viewed with some reservation given the poor predictive value of a dietary history.

Lactose intolerance

Lactose intolerance is the inability to digest lactose, a disaccharide found in mammalian milk. It is caused by a deficiency or insufficiency of lactase, and produces similar symptoms to IBS. Its diagnosis is made by a positive hydrogen breath test.

Lactose intolerance does not predispose one to IBS[80,81], but there may be an increased sensitivity to luminal distension by lactose in IBS patients.[82] Parker et al. documented lactose malabsorption in 27% of a cohort of 122 IBS patients.[83] The introduction of a lactose-restricted diet in those who tested positive did not yield any particular improvement in their symptoms. Despite the relatively high prevalence of lactose malabsorption (almost a third of their IBS patients), they questioned the validity of chasing this diagnosis, since its treatment did not offer symptomatic benefit.

Post-Infectious IBS

The observation that infection predisposes to IBS is well recognised.[84] This will be discussed at length in later chapters. The emergence of functional symptoms in someone with established inflammatory bowel disease has also been documented.[85-87] Peter Whorwell's group assessed 98 patients with

quiescent colitis, and noted that a third of them had symptoms consistent with IBS.[85] The mechanism for this may be that inflammation produces a up-regulation in visceral sensitivity and a change in sensorimotor function that persists even when the inflammatory infiltrate has retreated.[88,89]

Antibiotic-associated Diarrhoea

Careful history taking ensures that the timing of antibiotic exposure with the development of diarrhoea should leave little doubt as to the aetiology of the symptoms. Antibiotic-diarrhoea has a spectrum of disease severity ranging from nuisance diarrhoea to life-threatening colitis. Antibiotics produce a change in the gut microflora rendering it vulnerable to opportunistic pathological colonisation.

Primary Idiopathic Bile Acid Malabsorption

Primary idiopathic bile acid malabsorption (IBAM) causes IBS-like symptoms with diarrhoea being the predominant complaint. Many cases of IBAM have been misdiagnosed as diarrhoea-predominant IBS.[90] Studies show that between 30% and 50% of those with chronic unexplained diarrhoea have positive ^{75}SeHCAT tests.[91-93] A useful approach would be to offer a therapeutic trial of Cholestyramine to those patients with D-IBS who do not respond to standard therapy.

Endocrine Causes of Diarrhoea

Conditions such as carcinoid, thyrotoxicosis, medullary carcinoma of the thyroid, gastrinomas, VIPomas, glucagonomas and somatostatinomas may produce diarrhoea. They are rare conditions but may represent the driving pathology in someone labelled as being a refractory IBS patient.

Other Forms of Colitis

Colitis has many aetiologies including idiopathic colitis (Crohn's, Ulcerative Colitis or Microscopic Colitis), ischaemic colitis, vasculitis, toxic (chemical) colitis and infective colitis. Diagnosis relies on careful history, the acknowledgement of unusual symptoms that do not fit within the framework of the Rome criteria, endoscopic evaluation and reconsideration of alternative diagnoses in refractory IBS patients.

Intestinal Pseudo-Obstruction

This is a condition characterised by the occurrence of symptoms of intestinal obstruction in the absence of demonstrable organic obstruction. The clinical presentation may be acute or insidious. It is this latter group which may simulate IBS-type symptoms, particularly abdominal bloating.

Hirschsprung's Disease

Hirschsprung lent his name to the condition of congenital aganglionic megacolon in 1888. Diagnosis is usually made in the post-natal period with failure to pass meconium within 48 hours of birth, increasing abdominal distension, vomiting or neonatal enterocolitis

EXCLUDING THE DIFFERENTIAL DIAGNOSIS

History

The advantages of using an internationally-endorsed consensus-led instrument for the diagnosis of Irritable Bowel Syndrome are that there is increased physician confidence in the diagnosis and increased patient confidence in this dynamic diagnosis-making process. Given the absence of worrying features, there is a potential to minimise invasive and costly investigations, a benefit to both patient and state.

The presence of Rome 3-compliant abdominal pain and bowel dysfunction with no red flag symptoms should provide a clinician with ample conviction for diagnosis. Research has shown that such a clinical presentation will have a high positive predictive value for a diagnosis of IBS. If red flag symptoms are manifest, they have little discriminatory power in differentiating functional disease from organic disease.[42] Symptoms normally attributable to IBS may also be present (though not typical) in other disease states- Isgar demonstrated that a third of patients with ulcerative colitis in remission fulfilled the criteria for IBS.[85]

Examination

Examination of a patient with IBS will be mostly unremarkable, though comorbid medical signs may be elicited. The abdominal examination may reveal abdominal distension, the scars of previous procedures, for example diagnostic laparotomies, and generalised or focal abdominal tenderness. Abdominal tenderness must be carefully stratified according to its origin, emanating from either the anterior abdominal wall or the viscera. Carnett's sign produces increased focal abdominal tenderness (by asking the patient to contract the abdominal wall musculature) if abdominal wall pain is present.

Primary Care

The depth of investigation is determined by careful exposition of the history, taking into account age, atypical features, recent change in bowel habit, red flag symptoms and family history of organic disease (in particular colorectal cancer and inflammatory bowel disease). A routine set of blood tests including full blood count, liver function tests, inflammatory markers, thyroid function and serological markers for Coeliac disease (anti-endomysial antibodies or anti-tissue transglutaminase

antibodies) is an acceptable screening tool for suspected IBS. Family history of colorectal cancer with a change in bowel habit and anaemia would be an indication for faecal occult blood testing. Acute onset diarrhoea is presumed to be of an infective origin until proven otherwise by stool microscopy and culture.

Secondary Care

Tests to exclude more esoteric conditions are indicated. Investigations may include lactose tolerance testing, breath tests for small bowel bacterial overgrowth, urinary screening for laxative abuse and neuroendocrine screening. Radiological imaging can delineate slow-transit constipation with transit studies, and intra-abdominal or pelvic pathology using ultrasonography and CT scans. At this point in the investigative journey, recourse to endoscopy, employing either flexible sigmoidoscopy or colonoscopy should be considered.

The Value Of Diagnostic Testing

An important review published by Cash et al. addressed the value of diagnostic testing in patients who met symptom criteria for IBS.[94]

The results from the study are summarised as follows: there was a higher prevalence of Coeliac disease in the IBS population compared to the general population; there was no difference in the prevalence of colorectal cancer, inflammatory bowel disease, thyroid disease and lactose intolerance between the two groups; the multiplicity of tests inflicted on a patient including colonoscopy, ultrasound, stool ova and parasite testing, faecal occult bloods and hydrogen breath testing were largely redundant, given that they were unlikely to demonstrate organic disease.

Global approach to management

Evidence to support the comprehensive investigation of suspected IBS patients is limited. The approach favoured by the Rome Foundation is based on the idea of a positive diagnostic process, namely to identify and be confident in the symptomatology that supports the diagnosis of IBS. The traditional and time-honoured approach to dealing with a suspected IBS patient was to exclude organic pathology, and then, almost by accident, arrive at the diagnosis. This approach has now been discredited. The review by Cash et al. highlights how little the investigative process adds to the management of the patient.[94] A judicious use of selected investigations will reduce health care costs,

improve the confidence of the patient concerning the medical system and reduce potential complications arising from over-investigation.

The National Institute for Health and Clinical Excellence published guidelines on the management of IBS in February 2008.[95] The guidance recommended that the following tests should be performed in someone presenting with Rome-positive symptoms for IBS:

- Full blood count (FBC)
- Erythrocyte sedimentation rate (ESR) or plasma viscosity
- C-reactive protein (CRP)
- Antibody testing for coeliac disease (endomysial antibodies or tissue transglutaminase)

There was little evidence to support routine blood testing. However the costs of such tests are minimal. Furthermore the opportunity to be able to show patients that routine blood are normal was felt to be useful in maintaining a positive diagnostic process.

Investigations that were deemed to be *inappropriate* for the routine investigation of suspected IBS patients:

- Abdominal ultrasound
- Barium enema
- Rigid/flexible sigmoidoscopy
- Colonoscopy
- Thyroid function tests
- Faecal ova and parasite test
- Faecal occult blood testing
- Hydrogen breath test (for lactose intolerance and bacterial overgrowth)

The important caveat to this platitude for the non-investigation of suspected IBS patients is that any individual with red flag symptoms should be investigated in a manner appropriate to exclude serious organic pathology.

TREATING IRRITABLE BOWEL SYNDROME

Treatment of Irritable Bowel Syndrome is informed by a step-wise, progressive approach founded on a confident diagnosis. Vacillation and incoherence in the diagnostic process, a long and drawn out period of investigation and an illogical, haphazard prescription of therapy achieves very little. Repeated investigations undermine the confidence of the patient with the physician.[96] Education of the patient about the nature of the condition, and how their symptoms fit within the syndrome complex is paramount. O'Sullivan assessed a cohort of 70 adult IBS patients[97]: 77% felt that their knowledge of the condition was inadequate, with prevailing concerns centred on diet and cancer risk. Furthermore a significant negative correlation between perceived knowledge and length of hospital consulting was established. The study concluded that patient education could reduce health care utilisation.

During a consultation, the physician needs to be attuned to the concerns of the patient, and identify significant impairment in quality of life, adjusting treatment accordingly. Psychological comorbidity will alter the perception of somatic symptoms. The development of a robust doctor-physician relationship, with enquiry into daily stressors, problems at work, family or marital discord and recent bereavement have been shown to reduce health care costs and the number of medical consultations for IBS symptoms. A study by Owen et al. featured a review of the medical records of 112 patients with IBS.[98] The presence of documentation in the medical notes about psychosocial factors was viewed as evidence of a positive patient-physician relationship. Analysis of the data showed that such documentation was associated with decreased patient consultation over time.

CONSERVATIVE MANAGEMENT

Patient education may be tailored to the individual or it may take place in a group setting or as part of a multidisciplinary approach. Patients are more responsive to advice on changes to lifestyle, for example dietary advice, explanation, encouraging exercise, reassurance and stress management, than to discussion about pharmacological therapy.[99] Even with these simple and inexpensive interventions, the majority of patients will have satisfactory (though not complete) relief of symptoms. Relief of fear and the reassurance that symptoms are not attributable to cancer are important treatment goals. Thompson et al. assessed over 3000 primary care patients, identifying 76 patients with IBS.[100] Approximately half of the IBS patients were afraid that they had cancer.

Predictors for referral for specialist opinion were the denial of the role of stress, insistence on multiple tests and the frequency of defaecation.

Self-Management

Empowering the individual to deal with his or her own symptoms can be a constructive therapeutic strategy. Heitkemper allocated randomised IBS patients to either standard of care IBS management, a multi-component 8 session program (Comprehensive group) comprising weekly one hour sessions with a specialist nurse practitioner on a one to one basis, and a one off single session with a specialist nurse practitioner (Brief group).[101] The sessions featured advice on symptom awareness, reassurance, dietary counselling, relaxation techniques and cognitive-behavioural strategies. Each session was tailored to the individual. Homework was assigned at the end of each session, re-presented at the next session, and specific symptom management plans were constructed. 12 month follow-up demonstrated significantly improved psychological and quality of life scores (SCL-90, IBSQOL) and reduced abdominal pain and functional impairment compared to those managed using the standard of care approach.

Multidisciplinary Team

The role of the multidisciplinary team was assessed by Saito et al. by comparing a group of IBS patients who attended a one day multidisciplinary group program of IBS education with a group of patients who did not attend.[102] At 6 months follow-up, 29% of those attending the IBS class did not meet the Rome criteria for IBS at 6 months follow-up compared to 11% of the non-attenders. Class attendance predicted higher odds of not meeting the Rome criteria at follow-up. Class attendance was associated with an improvement in Health-Promoting Lifestyle Profile scores on multivariate analysis, though interestingly, it was not associated with change in pain, quality of life, satisfaction, or health care utilisation. The authors concluded that the multidisciplinary educational approach might be a model of care that should be incorporated into routine clinical practice.

Diet and Exercise

The symptoms of Irritable Bowel Syndrome may be exacerbated by a variety of dietary factors. The unremitting identification of dietary factors that contribute to symptom generation, and the subsequent elimination of said products from the diet may significantly improve the clinical

presentation. No single, uniform diet can be ascribed to the IBS population. Therefore the individual must be encouraged to identify for him or herself the foodstuffs that cause and prolong symptoms.

Many people with IBS are said to have food intolerance. This is terminology which must be differentiated from true immunologically-mediated food allergy. The central tenet of food intolerance is that withdrawal of the foodstuff produces a resolution of symptoms whilst reintroduction will prompt recurrence.

Fibre

Dietary fibre comprises plant-derived non-starch polysaccharides such as cellulose and other plant components. Fibre is not digested by the gastrointestinal tract, and moves steadily through the digestive system, absorbing water, contributing to the faecal bulk and easing defaecation. Research assessing the use of fibre in the treatment of IBS is conflicted, largely due poor study methodologies and high placebo responses. Furthermore, some types of insoluble fibre may exacerbate symptoms. The traditional approach of the gastroenterologist has been to advocate the increased intake of fibre to improve transit time.[103] Studies assessing fibre intake in IBS have produced little favourable evidence in the support of this approach, and some studies report a worsening of symptoms if fibre is prescribed.[104,105]

Starch

Resistant starches are large carbohydrate polymers that are poorly absorbed by the stomach or small intestine. By definition, starch that manages to travel undigested all the way through the proximal and mid gut to the colon is termed a resistant starch. Reduction in resistant starches may produce an improvement in bloating and excessive flatus.

Lactose

IBS patients commonly describe lactose intolerance. A careful elimination of lactose and subsequent reintroduction may be indicated in those patients who complain of diarrhoea and excessive flatus after lactose ingestion. However, it must be noted that reported lactose intolerance correlates poorly with true lactose malabsorption. Suarez et al. demonstrated this beautifully by giving either 240 ml of lactose-containing or lactose-deficient milk to subjects who reported severe lactose intolerance.[106] There was no statistical difference in symptomatology (bloating, abdominal pain, diarrhoea and flatus) for when lactose was present or absent.

Exercise

Physical activity has a significant impact in reducing the risk of major disease including ischaemic heart disease, stroke and hypertension. Exercise can alleviate the symptoms of musculoskeletal pathology, e.g. back pain and osteoporosis, and mood dysfunction, e.g. dysthmic states and anxiety.

Laxatives

Constipation-predominant IBS patients gravitate towards increased dietary fibre or laxatives to improve bowel habit. Stimulant laxatives are not recommended in IBS owing to their erratic mode of action and side effect profile (e.g. tachyphylaxis). Inorganic salt-based laxatives (e.g. polyethylene glycol) are better tolerated than organic alcohols and sugars (e.g. Lactulose) which may exacerbate bloating and flatulence.[107]

Aloe Vera

A randomised double blind placebo-controlled trial by Davis et al. failed to discern a therapeutic benefit of treating IBS patients with Aloe Vera over placebo.[108]

Complementary and Alternative Medicine

Complementary medicine is widely used by the IBS population. van Tilburg et al. reported that alternative medicines were used by 35% of a population of patients with functional bowel disorders with a median expenditure of $200 per patient.[109] Increased use of complementary therapies was associated with higher education, anxiety, younger age and the female gender.

Acupuncture

Acupuncture has been shown to short term relief of symptoms in IBS, although controversy rages as to whether it is nothing more than a placebo response. Schneider et al. published a study that randomised IBS patients to acupuncture or sham acupuncture (using the Streitberger needle).[110] After ten sessions of therapy, both groups showed improvements in quality of life from baseline, assessed using the functional digestive diseases quality of life questionnaire (FDDQL). There was no statistical difference between the two groups, endorsing the role of the placebo response.

Reflexology

Tovey et al. were responsible for the only study of reflexology in IBS.[111] Thirty-four patients were randomised to receiving reflexology-congruent foot massage or just a foot massage. Reflexology did not improve any of the symptoms assessed.

MEDICAL THERAPY

Anti-motility drugs

Anti-motility drugs are a useful therapy for patients with diarrhoea-predominant IBS. Changes in small and large bowel contractility, increased intestinal secretions and reduced intestinal absorption may produce a diarrhoea-predominant clinical picture. The main categories of anti-motility drugs used in IBS are Loperamide, Co-phenotrope, Codeine Phosphate and Morphine-containing compounds

Loperamide

Loperamide is an opioid receptor analogue. Its main site of action is the μ-opioid receptors in the myenteric plexus of the large bowel. It does not cross the blood brain barrier, and therefore does not have the same stimulatory effects on the central nervous system as other opioid analogues. The clinical effect of Loperamide is to reduce colonic peristalsis and secretion, resulting in longer gastrointestinal transit time and increased absorption of fluids and electrolytes from the gastrointestinal tract.

Co-phenotrope

Co-phenotrope is composed of 2 pharmacologically active compounds Diphenoxylate and Atropine. Diphenoxylate is responsible for the anti-diarrhoeal properties[112-114]

Antispasmodics

Antispasmodics are targeted at the visceral dysmotility and enhanced colonic contractility seen in IBS patients, particularly of the diarrhoea-predominant subtype. Anti-spasmodics have been subject to a proliferation of clinical research and meta-analysis.[115-119] Poynard's 2001 meta-analysis assessed 23 randomised clinical trials that investigated anti-spasmodic therapy in IBS. The mean percentage of patients responding to the anti-spasmodics was 56% compared to 37% in the control groups when looking at global symptom scores. Abdominal pain was improved in 53% of patients in the active groups compared to 41% in the placebo groups.

Probiotics

Emerging data suggests the clinical utility of probiotics in IBS. The research that has been carried out in recent clinical trials has been constrained by small study numbers and differences in the strain of

probiotic used, the duration of treatment and the study design. Some studies have used a single bacterial isolate whereas others have used a cocktail of strains making interpretation of data difficult. Further studies need to be conducted into the use of probiotics in IBS, principally trying to identify the optimum strain, dose and duration of therapy. At present the mechanism of action of probiotics can only be surmised.

Antidepressants

Antidepressant therapy in IBS is primary aimed at harnessing the analgesic rather than the anti-depressant properties of the medication. It is necessary to make this distinction clear to patients in order to improve compliance.

Tricyclic Antidepressants

Tricyclic antidepressants (TCAs) are a popular choice for refractory IBS symptoms with the predominant action being to reduce abdominal pain.[120] Studies that have attempted to demonstrate an improvement in visceral hypersensitivity by using TCAs have not been so convincing.[121] Over 10% of IBS patients are subjected to a trial with a TCA.[122] Many studies support the superiority of low-dose TCAs over placebo in IBS.[123]

Selective Serotonin Reuptake Inhibitors

Selective Serotonin Reuptake Inhibitors (SSRIs) are a newer class of anti-depressant compared to the TCAs, and benefit from having a much more specific pharmacological action. In the context of IBS, selective Serotonin reuptake inhibitors have been the subject of four randomised control trials.[124-127] All four studies seemed to suggest an improvement in overall well-being on an SSRI.

PSYCHOLOGICAL THERAPY

Self-Help Groups

Simple interventions such as providing self-help guidelines for patients with IBS can be effective in terms of symptom relief and cost benefit. Robinson et al. randomised IBS patients to receive a self-help guide on its own, to receive the guide in conjunction with a self-help group session or to receive none of the interventions (control group) in a primary care setting.[128] One year follow-up showed a 60% reduction in primary care consultations and decreased perceived symptom scores in the IBS cohort that received the both the self-help guideline and the group session. Clearly, patient education must be integral to the management paradigm, although IBS patients may have little

understanding of their condition.[129] This approach when combined with rational advice about modifying lifestyle measures (including diet) allows patients to assume a degree of mastery over their symptoms. Effective coping and quality of life are nurtured by the development of shared management between the patient and physician.[130,131] Patients who continue to attribute their symptoms to undiagnosed organic disease are predisposed to multiple consultations in primary care and subsequent referral to secondary care.

Relaxation Therapy

Stress is identified by many IBS patients as a potent exacerbant of symptoms. The role of relaxation therapy is aimed at reducing the burden of daily stressors to reduce symptomatology. Forbes et al. compared the use of a simple relaxation tape with hypnotherapy in a group of refractory IBS patients over a 12 week prospective study period.[132] The study proposed that hypnotherapy was the superior treatment. However, the audiotape still demonstrated a valuable therapeutic effect, and therefore should be considered as a viable second-line treatment in resistant IBS.

Biofeedback

Biofeedback is a multi-component discipline that incorporates patient education, relaxation therapy, thermal biofeedback treatment and the development of coping strategies for daily stressors. Biofeedback has been shown to be more effective than symptom monitoring in the treatment of IBS although other factors may be just as useful.

Hypnotherapy

Hypnotherapy has become an important treatment modality for refractory Irritable Bowel Syndrome. A landmark study by Whorwell et al. in 1984 documented a clear improvement in symptoms scores in patients treated for 3 months with hypnotherapy compared to those treated with psychotherapy and a drug placebo. A systematic review in 2005 consolidated the proliferation of research in the intervening years that was concerned with the clinical utility of hypnotherapy in IBS.[133] Fourteen studies were identified, of which 8 featured control groups. The review concluded that hypnotherapy consistently improved both colonic and extra-colonic symptoms in the majority of IBS patients. According to the standards imposed by the Clinical Psychology Division of American Psychological Association, “the use of hypnosis with IBS qualifies for the highest level of acceptance

as being both efficacious and specific". Of all the psychological therapies available to IBS, hypnotherapy is the most extensively researched medium.[134]

Hypnotherapy is most effective when performed with a therapist, although self-taught hypnotherapy tapes have become widely available.[135] A recent study has established that the effects of hypnotherapy may persist even at 5 year follow-up.[136] The mechanisms that drive symptomatic relief are not known. Apart from the moderation and manipulation of psychological processes, hypnotherapy has been shown to normalise disordered rectal sensitivity,[137,138] and to alter colonic motility. IBS-cognitions and health-related quality of life improve in tandem with the improvement of colonic symptoms.[139]

Hypnotherapy is expensive, time-consuming and poorly available. A typical treatment regime comprises 12 weekly sessions with a trained therapist. Evidence for the long-term persistence of symptomatic benefit after hypnotherapy and the reduction in drug and consultation costs produce powerful arguments for its use in refractory IBS patients. Research has started to identify which patients will be most amenable to hypnotherapy. Whorwells's group published a recent abstract suggesting that if a patient perceives his or her illness as visual representation, then they will be more likely to respond to hypnotherapy than patients who use literary or visceral abstractions. Hypnotherapy seems to benefit all subtypes of IBS with the curious exception of diarrhoea-predominant males.[140]

Cognitive Behavioural Therapy

Cognitive behavioural therapy (CBT) is founded on the concept of social learning theory, originally expounded by the work of Gabriel Tarde in the 19th century. This states that behaviour is a reaction to social observation and social consequence leading to the development of new imitatory behaviour. As an example, a particular behaviour may induce increased attention on that individual, thereby promoting that behaviour through reinforcement. Cognitive behavioural therapy targets specific behavioural patterns, focusing on a cognitive approach to amplify or reduce that behaviour. In the context of IBS, CBT can highlight abnormal illness cognitions and chronic pain behaviour.[141] Cognitive behavioural therapy may be effective in reducing co-existing psychological symptoms in IBS patients, notably anxiety and depression.[142] Furthermore, as a general point, CBT may enable patients with chronic disease to have better coping mechanisms.[141]

Cognitive behavioural therapy is usually not a single specific therapy. Many centres recommend a multi-component approach with an amalgamation of cognitive and behavioural interventions, stress-management, relaxation therapy, pain management and assertiveness training. The ultimate goal is to address behaviour that impacts on bowel habit and to reduce the psychosocial distress that it causes.

The first study to assess CBT in IBS was performed by Bennett and Wilkinson in 1985.[143] Twelve IBS patients underwent an 8 week multi-component plan (stress-management training, cognitive therapy and contingency management) in contrast to 12 patients assigned to best medical care (fluphenazine/nortriptyline with an anti-spasmodic). Anxiety levels were reduced in the treatment group. Both groups demonstrated a similar improvement in gastrointestinal symptoms, fatigue and functional impairment. Since this initial work, the studies investigating CBT in IBS have become more sophisticated and better powered. Cognitive behavioural therapy has been established as superior treatment to conservative management or self-help support groups.[144,145]

Toner et al. published a series of 101 IBS patients randomised to either CBT, attentional-placebo control or best medical care.[141] Symptoms of depression and bloating improved compared to controls. Scores reflecting greater patient satisfaction with therapy were higher in the CBT group compared to controls.

Drossman et al. enrolled 431 IBS patients into a randomised comparator-controlled, multi-centre trial pitting CBT against education, desipramine and placebo.[120] The patients were female with a moderate to severe disease severity. Intention-to-treat analysis demonstrated that CBT was significantly more effective than the education group unless there was co-existent depression (70% response rate compared to 37%); desipramine was of no benefit compared to controls on an intention to treat analysis, but showed benefit when using a per-protocol analysis. The aim of this study was to assess the efficacy of CBT against an education control and desipramine against placebo- there was no design to directly compare CBT to desipramine. Furthermore CBT seemed to improve global satisfaction and quality of life rather than abdominal pain.

Psychodynamic Interpersonal Psychotherapy

Psychotherapy explores the complicated interpersonal problems that patients have experienced. A common example may be childhood trauma or upset which precipitates behaviours and cognitions

that persist into adulthood. It is the responsibility of the therapist to identify these areas of distress, to gently stimulate discussion and to allow time for reflection. During the sessions the patient may reproduce anxieties around the areas of conflict which induce IBS-like symptoms. By revisiting these topics, a clear progression and link can be established between psychological distress and symptom generation. This allows the patient to develop a greater understanding about what is causing his/her symptoms. With practise the patient can develop the skills required to minimise the psychological burden in order to improve the bowel habit.

Psychotherapy can be a profoundly time-consuming process featuring weekly or fortnightly sessions for a few years. Short courses of psychotherapy may also be instigated featuring between 10 and 20 sessions that specifically focus on one particular aspect of a patient's behaviour, termed focal psychotherapy. Psychotherapy also demands a robust relationship between patient and therapist, and the motivation of the patient to undergo such a time-intensive intervention.

Psychotherapy has been shown to be effective in treating IBS patients refractory to conventional medical therapy.[124,146,147] The methodological sanctity of some of the earlier studies assessing psychotherapy in IBS has been questioned.

A large study conducted by Creed et al. randomised IBS patients to 8 weekly sessions of psychotherapy, 20mg of Paroxetine daily or routine medical care.[124] Both the active treatments were found to be effective- increased scores for the physical aspects of health-related quality of life were seen in both groups although psychological scores remained non-significant. Interestingly at 1 year follow-up psychotherapy was associated with reduced health care costs (not seen in the Paroxetine group), suggesting that even a brief (8 week) trial of psychotherapy is clinically and cost-effective. A further point to draw from this study is that the improved health-related quality of life scores seen in the psychotherapy group did not significantly improve abdominal pain scores, and the positive therapeutic differences could not be entirely attributable to changes in psychological profile.

Psychotherapy has been demonstrated to increase tolerance to rectal balloon distension.[148] Balloon distension using a barostat is a technique used as a biomarker for visceral hypersensitivity. However, the increased tolerance may be secondary to improved psychological status (e.g. improved depression scores) rather than a change to primary visceral afferent nociceptive signalling. Psychotherapy has also been found to be particularly effective in IBS patients who report a history of sexual abuse.[149]

CHAPTER ONE, SECTION 2:

PATHOGENESIS OF IRRITABLE BOWEL SYNDROME

The mechanisms that underlie IBS are poorly understood. The current understanding is based on the concept of the Brain Gut Axis which describes the interaction between central (e.g. sensory cortex, central pain centres, hypothalamic-pituitary adrenal axis) and local structures (e.g. colonic mucosa and sensory afferent nerve fibres). The Brain Gut Axis determines how the psychological state and the stress response influence the perception of painful and non-painful stimuli from the gut. Pathophysiological mechanisms which may influence pain perception from within the gut wall include colonic dysmotility, visceral hypersensitivity, abnormal fermentation, post-infective bowel dysfunction and immune activation.

PSYCHOPATHOLOGY

Chaudhary and Truelove described the condition of Irritable Colon Syndrome, subsequently to be renamed Irritable Bowel Syndrome, in their landmark study in 1962.[84] One hundred and thirty patients were identified with recurrent abdominal pain and disordered bowel habit with no evidence of organic pathology that could explain their symptoms. The cohort of patients was subdivided according to prevailing bowel habit- the spastic colon group and the painless diarrhoea group- and the raw demographics data was presented. The importance of this paper was that no only did it give a name and prominence to a syndrome that had perplexed the medical profession for many years- terms such as “membranous enteritis” and “neurogenic colitis” had been proposed by earlier authors- but it also sought to identify predisposing factors to the condition. Chaudhary and Truelove demonstrated that psychological factors were “incriminated” in more than 80% of the patients, that females were more likely to have psychological comorbidity, and that prominent psychological factors predicted a worse prognosis.

Patients with IBS are more likely to use health care resources and to undergo comprehensive investigation to exclude organic disease. IBS patients with psychological comorbidity are more likely to present for repeat medical consultations than IBS patients who are psychologically unaffected. Female IBS patients in primary care are more affected by the physical and psychosocial symptoms than their male counterparts. These findings propose a number of ideas about the pathogenesis of IBS. The psychiatric or psychosocial component that seems so prominent in IBS may be completely

unrelated to the abdominal symptomatology. It is important to note that the presence of psychological comorbidity in a patient with abdominal symptoms does not imply causation. So, in effect, the psychosocial burden may merely be a concurrent, though hardly innocent, bystander. Secondly, chronic abdominal pain, the constant need to take refuge in the lavatory and the social embarrassment and stigma of the condition may conspire to cause distress and psychological dysfunction. The final explanation that IBS is primarily a psychiatric disease that manifests in somatic symptomatology. Researchers have looked at this mechanism, by comparing patients with psychiatric disease to IBS patients with psychological comorbidity, and found striking similarities between both groups in terms of psychological and somatic function. Additional weight to this particular argument is that the era of psychological intervention is now firmly established for treating IBS patients with refractory symptoms. Methods such as cognitive behavioural therapy, psychodynamic interpersonal psychotherapy and behavioural therapy are valid, viable and increasingly popular treatment options.

Irritable Bowel Syndrome may represent a disparate and heterogeneous group of patients who are grouped together solely by virtue of the fact that they have abdominal pain. Variations in psychiatric or psychological comorbidity will therefore reflect the differing underlying aetiologies. If a primary psychogenic process is dominant in the generation of IBS symptoms, this may represent a central somatoform-type presentation. In other words, the psychological state is outwardly represented in the language of somatic symptomatology. There is some evidence of this in the literature. Levy et al. documented that 78% of the extra visits to a medical practitioner by over-attending IBS patients are for non-gastrointestinal symptoms. If a non-psychiatric process is dominant, one would expect IBS to share a common and unique aetiology with related disorders. Whitehead et al. addressed the issue of whether IBS patients were at risk of specific comorbid disorders or whether IBS patients elaborated and over-reported their symptoms. A cohort of 3153 IBS patients was compared to age and gender-matched controls and a cohort of patients with Inflammatory Bowel Disease. Patients were categorised into whether they had gastrointestinal, psychiatric or non-gastrointestinal somatic complaints. The non-gastrointestinal somatic group was sub-divided into whether diagnosis was achieved through symptom-based or biomarker-based methods. The results showed that IBS were more likely to have concomitant somatic disorders than controls and that somatic comorbidity was positively correlated with psychiatric comorbidity. However there were no unique associations between IBS and the somatic disorders. The authors concluded that IBS patients experienced

disordered symptom perception and pain amplification rather than any shared pathophysiology, and that psychiatric comorbidity could influence but not entirely explain somatic comorbidity.

Abnormal Illness Behaviour

The repeated presentation of IBS patients to medical services may be related to abnormal illness behaviour. This describes the relationship of the patient to his or her prevailing symptomatology. Changes in symptom perception regardless of any objective change in clinical presentation may be sufficient to produce health-care seeking behaviour. Negative cognitions about one's symptoms, worries about the future and a constant fear that abdominal symptoms are harbingers of a cancerous lesion are consistently reported in the IBS literature. In addition to seeking out medical services, abnormal illness behaviour may take the form of hypochondriac beliefs, disorders of affect, denial and the conviction of undiagnosed disease.

Abnormal illness behaviour was first described by Pilowsky in 1969.[150] His definition described "the persistence of a maladaptive mode of experiencing, perceiving, evaluating and responding to one's health status, despite the fact that a doctor has provided a lucid and accurate appraisal of the situation and management to be followed (if any), with opportunities for discussion, negotiation, and clarification based on adequate assessment of all relevant biological, psychological, social and cultural factors".[151] His definition was an abstraction of the concept of illness behaviour, as proposed by Mechanic.[152] This states that when an individual becomes unwell and assumes the sick role,[153] a series of adaptive (or maladaptive) behaviours ensues, differentiating the well individual from the unwell individual. This change of behaviour is designed to promote a return to health. However deviant behaviour may encourage a pathological protraction of the sick role, namely abnormal illness behaviour.

The development of abnormal illness behaviour may result from a disordered assimilation of witnessed behaviours in childhood. IBS patients adopt abnormal illness behaviour primarily through two mechanisms- the adoption of the sick role and the modelling of illness behaviour from parents. Levy et al. illustrated this latter point when he reported that the children of IBS patients consulted medical services far more than children of non-IBS patients, both for gastrointestinal and non-gastrointestinal complaints.[154] By asking patients about the symptoms they experienced in childhood and the number of visits they made to the doctor, IBS patients recollect significantly greater bowel

dysfunction, abdominal pain and healthcare utilisation when compared to patients who did not go to see their doctors (non-consulters) and asymptomatic controls.[155]

Whitehead et al. compared IBS patients with patients with peptic ulcer disease.[156] The data showed that IBS patients were more likely to have a multiple somatic complaints, to view their symptoms as being more significant than other people and to visit the doctor with increased frequency for minor illnesses. IBS patients were more likely to have had 2 or more colds during the past year, that their colds were more serious than those of other people and that they would rather attend a medical consultation than attempt to get better by themselves. There were no differences between the two groups with regards to depression or anxiety. IBS patients were more likely to have been given treats such as sweets, toys, gifts or food (such as ice cream) during episodes of sickness. The direct reinforcement of somatic complaints using a reward system was compelling evidence of learned illness behaviour in IBS.

Another explanation for the observation that IBS patients beget IBS children may be a due to the fact that there is a genetic or hereditary factor. Levy assessed the prevalence of 80 health disorders (one of which was IBS) in populations of monozygotic and dizygotic twins. Concordance of IBS was significantly higher in monozygotic twins than the dizygotic twins (17.2% vs. 8.4%) favouring a genetic lineage. However, the proportion of dizygotic twins with IBS who had mothers with IBS (15.2%) was greater than the proportion of dizygotic twins with IBS who had co-twins with IBS (6.7%). A parent with IBS was an independent predictor of IBS, and that this association was stronger than having a twin with IBS. The authors concluded that although genetic factors were important, social learning of abnormal illness behaviours passing from parent to child were more powerful influences.

Whitehead demonstrated that there was a positive correlation between childhood reinforcement of the sick role and the development of abnormal illness behaviour in later life, even when significant confounders such as neuroticism and stress had been accounted for.[157] Another curious observation was that abnormal learning behaviour was a specific phenomenon- childhood menstrual symptom reinforcement predicted abnormal menstrual illness behaviour but not abnormal illness behaviour for other symptoms, such as cold illness behaviour or bowel illness behaviour.

The relationship between how parents deal with their own symptoms and the somatic complaints of their children has a significant bearing on the development and maintenance of symptoms in

adulthood. This is important because it suggests that recognition and resolution of childhood reinforcement may prevent adult comorbidity. A fascinating insight into social learning was provided by the observation that a child would be more likely to develop recurrent abdominal pain if his (or her) mother suffered from abdominal pain when compared to the father having abdominal pain. The inference is that a child spends much more time in the company of the mother during those impressionable years than the father.[158,159] This may have far-reaching repercussions- 8% of children experience recurrent functional abdominal pain, and of these 18-61% will go on to develop abdominal pain or IBS 5-30 years later.

Koloski et al. investigated the relationship between abnormal illness behaviour and health care seeking in IBS and functional dyspepsia.[160] Powered by the Abnormal Illness Behaviour Questionnaire and the Social Learning of Illness Behaviour scale, this study consulted a representative sample of the Australian public using a postal survey. Contrary to the authors' expectations, the parameters for abnormal illness behaviour did not differentiate between IBS consulters and IBS non-consulters. The unshakeable conviction that a patient has serious disease despite every reassurance to the contrary (assessed using the Disease Conviction scale) emerged as the only independent predictor of having a diagnosis of IBS. Their conclusion was that IBS patients were characterised by the belief that significant pathology was present, but that this did not reflect in health care seeking. This study sits at odds to other research that has demonstrated a link between abnormal illness behaviour and health care seeking in IBS patients.[61] Previous work by Talley et al. could not provide an association between health care seeking in IBS and psychological factors (including neuroticism) or abuse history.[161] Only increased duration of abdominal pain and increased pain severity were independent predictors of health care seeking.

Research that focuses on childhood symptomatology in retrospective studies is vulnerable to recall bias. Collateral history from parents is not usually available. Furthermore the methodology of self-report studies when investigating abnormal illness behaviour may be inherently flawed. A solution to this is to construct a prospective study assessing childhood symptoms, illness behaviour and healthcare seeking. Howell et al. managed to achieve this by studying a prospective longitudinal birth cohort.[162] The study demonstrated that IBS at the age of 26 years was significantly more common among individuals with a history of chronic abdominal pain between the ages of 7 and 9. This was assessed by clinical history (OR: 1.85, P=0.02), parental report (OR: 1.82, P= 0.03) and health care consultations (OR: 3.75, P= 0.03).

Abnormal illness behaviour must be viewed in the context of a patient's subjective appreciation of their symptoms. This is formed from personal belief systems, childhood experiences, knowledge of the disease processes and various cultural and social references. Gick et al. assessed a group of university students to determine whether one's perception of illness had any relationship with health care seeking.[163] Whilst there were no differences in neurotic traits between students with IBS who consulted their doctors compared to those students who did not consult, the significant finding was that students who were bothered about their symptoms and who questioned the meaning of their symptoms were more likely to seek health care services. A study by Kettel et al. confirmed that anxiety about the potentially serious nature of a patient's IBS symptoms (and symptom severity) differentiated IBS consulters and from IBS non-consulters.[63] Similar studies have confirmed that anxiety related to the prevailing symptoms and the fear of serious disease acts as an independent predictor for healthcare seeking.[164]

BIOPSYCHOSOCIAL MODEL OF FGIDS

Classical psychosomatic theory suggested that a common psychologically-mediated pathogenetic mechanism was responsible for many medical disorders in the early 1950s. Such disorders included asthma, allergy, false pregnancy, coeliac disease, peptic ulcer and migraine. That psychological dysfunction was the primary mechanism of symptom generation was based on the fact that the popular implementation of psychoanalysis had a beneficial therapeutic effect- ergo, all conditions must have at their heart emotional conflict. This concept was quickly discredited. Its demise prompted a radical shift to the opposite end of the spectrum, introducing an era of scientific research that sought to discover various biological markers for the functional disorders. This was termed the biomedical model of medicine.

In 1977, Engel proposed the biopsychosocial model.[165] This was a reaction to the biomedical model that attempted to explain every characteristic of disease presentation by pathological abnormality. Engel argued that disease phenotype was the fusion of biological processes, psychological state and social factors, thereby creating a holistic approach to disease conceptualisation. The biopsychosocial model was readily embraced because it could start to explain the relationship between an individual, his body, his emotions and his environment. It also allowed the development of sensitive psychological and psychosocial instruments to be constructed in order to assess psychological distress and social context in the generation of functional disorders.

MICRO-INFLAMMATION

POST-INFECTIOUS IRRITABLE BOWEL SYNDROME

The prevalence of Post-infectious Irritable Bowel Syndrome (PI-IBS) is as high as 17% in the United Kingdom, although there seems to be a wide geographical variation with reported prevalence as low as 6% in US tertiary care.[166] Post-infectious Irritable Bowel syndrome represents a subgroup of patients who were asymptomatic until the index episode of gastroenteritis. During this phase, the immunological insult from the enteric pathogen induces a process of visceral sensitisation. The patient begins to experience the symptoms of IBS in the post-acute setting, and these symptoms may persist indefinitely. Rodruigez et al. proposed a relative risk of 11.9 of developing PI-IBS in the 12 month period after an episode of bacterial gastroenteritis.[167] Risk factors for the development of PI-IBS include the severity of the index infection (including prolonged fever), bacterial toxigenicity, younger age, being female and various psychiatric co-morbidities including neuroticism, anxiety, depression and hypochondriasis.[168,169]

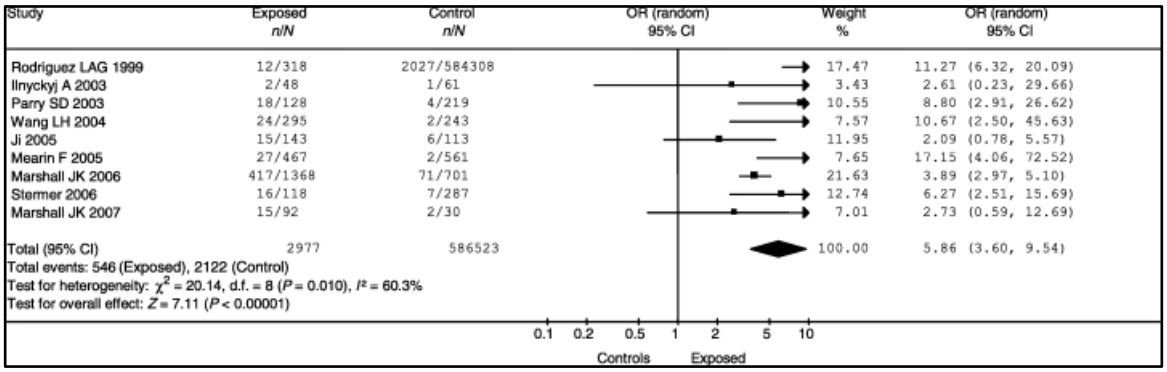
The link between enteric infection and post-infectious abdominal pain and bowel dysfunction (what we would now term PI-IBS) started to emerge at the end of the second world war- British troops returning from the African theatre of war complained of persistent symptoms following on from amoebic dysentery.[170] The clinical course of IBS varies from individual to individual. A prospective study was undertaken by Okhuysen et al. which observed North American students on their travels to Mexico.[171] One hundred and sixty-nine students entered the trial and completed gastrointestinal questionnaires during the 5 week stay in Mexico. They were followed up for 6 months on their return back to the United States. 63% reported episodes of diarrhoea in Mexico, mostly due to E. coli sepsis. Among the affected students, persistent symptoms were seen at 6 months, including diarrhoea (18%), abdominal pain (18%) and frequency (9%). 10% of the infected students met the criteria for PI-IBS. These findings substantiated an earlier prospective case-controlled study by Parry et al.[172] This study reported an incidence of PI-IBS (using Rome 2 criteria) in 16.7% of the patients who had laboratory-confirmed gastroenteritis, and functional diarrhoea in six other cases (5.6%). The odds ratio for acquiring IBS following bacterial infection was 10.1 (95% CI = 3.32-30.69) compared to controls. Follow-up at 3 month and 6 month time points remained consistent between the two groups.

The medical literature reports numerous studies investigating different species of enteric pathogen. The largest study on PI-IBS featured stool-positive Campylobacter jejuni infection (Reference). Of the 840 patients were enrolled, 13.8% (103 patients) fulfilled Rome 1 criteria for IBS after the gastroenteritis. It is important to note that those patients with pre-existing IBS (numbering 46 in total) were excluded from these results. The incorporation of Rome 2 sub-typing identified diarrhoea-predominant IBS as the most common form (63%) compared to constipation-predominant IBS (13%) and alternating sub-type (24%). Reference.

Other bacterial pathogens may precipitate PI-IBS including Salmonella[173,174], and Shigella.[175] This latter study reported an odds ratio of developing PI-IBS after Shigellosis of 2.09, somewhat lower than other studies, and discovered that the duration of diarrhoea was an independent risk factor of PI-IBS. A 3 year follow-up of the same patient population demonstrated that 25% returned to a normal bowel habit.[176] A recent Turkish study has implicated Trichinella in PI-IBS.[177] Indeed, the increased risk of PI-IBS following bacterial appears non-specific- single species studies and dual infection studies (such as the co-infection of Campylobacter and E. coli in a large waterborne outbreak of bacterial dysentery[178]) yield similar conclusions. Viral gastroenteritis is a much more common phenomenon, displaying a more benign clinical course. Its role in the development of PI-IBS is thought to be less potent than bacillary gastroenteritis.[179] This observation is substantiated by meta-analysis by Halvorson et al. which demonstrated that the risk of PI-IBS was higher in bacterial gastroenteritis compared to non-bacterial gastroenteritis, and that bacterial gastroenteritis was associated with a more severe symptomatology (vomiting, fever, abdominal pain and diarrhoea).[180]

Meta-analysis of the incidence and prognosis of PI-IBS by Thabane et al. concluded that there is about a 6-fold increased incidence in PI-IBS following bacterial infection.[169] This was computed from pooled data from 9 published prospective trials. Figure 1.2.1 shows this meta-analysis.

Figure 1.2.1 Incidence of Post-infectious Irritable Bowel Syndrome



Adapted from M. THABANE, D. T. KOTTACHCHI & J. K. MARSHAL, Alimentary Pharmacology & Therapeutics Vol. 26, 4 Pages: 535-544

An important study by Spence et al. investigated the psychology of PI-IBS.[181] 620 primary care patients who were stool-positive for Campylobacter with no preceding history of IBS or significant bowel pathology were recruited into a prospective study. Psychometric questionnaires were competed at the beginning of the study. These questionnaires were validated instruments for assessing levels of stress, mood, perfectionism, negative illness beliefs and negative illness behaviours. 49 patients met the criteria for IBS at 3 and 6 months follow-up. Those who developed PI-IBS were found to have significantly higher levels of stress, anxiety, somatisation and negative illness beliefs at the time of infection compared to those patients who did not develop IBS. Furthermore they were more likely to remain active despite their symptomatology until they were forced to rest, displaying “all-or-nothing behaviour”. Spence et al. have raised a fascinating insight into the pathogenesis of PI-IBS- enteric infection is by definition the trigger for symptom-onset, but psychopathology may be the propagating factor. An earlier study by Dunlop et al. demonstrated that depression and increased enterochromaffin cell counts were independent risk factors for the development of PI-IBS.[182]

Inflammation in Irritable Bowel Syndrome

The observation that enteric infection predisposes to IBS has resulted in research looking for an immunological explanation for symptom generation. Studies in the last five years have demonstrated that IBS patients may have raised levels of inflammatory mediators. Various approaches for the investigation of inflammation in IBS have been used including colonic mucosal sampling, peripheral blood mononuclear cell (PBMC) sampling and PCR techniques. A novel technique uses the mucosal patch technique- this comprises the placement of cellulose patches adjacent to the rectal mucosa, secured by rectal balloon.[183] Inflammatory mediators are evolved from the mucosa and then absorbed by the patches. The study neatly demonstrates markedly raised inflammatory mediators (myeloperoxidase from neutrophils; eosinophil cationic protein from eosinophils) in active Ulcerative Colitis, and raised levels in IBS patients compared to controls. Interestingly, the levels that were seen in the IBS patients were equivalent to those acquired from patients with inactive Ulcerative Colitis.

Schoepfer et al. evaluated the recent introduction of faecal inflammatory markers showing encouraging sensitivities and specificities for differentiating IBS from Inflammatory Bowel Disease- see earlier references to this study.[184]

Cytokines

The increasingly prolific use of cytokine analysis in IBS has presented data suggesting that low-grade inflammation may represent an important pathogenetic mechanism. Dunlop et al. demonstrated increased enterochromaffin cell (EC) density and lamina propria T cell lymphocytes in rectal mucosal biopsies taken from PI-IBS patients compared to active controls and healthy volunteers.[182] Animal studies have shown that EC hyperplasia is dependent on T cell activity- specific T cell deficiencies can be artificially recreated using T cell knockout mice, resulting in decreased EC hyperplasia and infection-induced mastocytosis.[185]

Wang et al. confirmed the increased expression of IL-1β mRNA in recto-sigmoid and terminal ileal mucosal biopsies taken from PI-IBS patients compared to controls.[186] This particular study also demonstrated increased mast cell density in the ileal biopsies in both PI-IBS and non-PI-IBS patients compared to controls, with increased nerve fibre staining (using specific stains for neurone specific enolase, substance P, 5-hydroxytryptamine and calcitonin gene related peptide) seen around mast

cell clusters. This data suggests a complicated relationship between immunological factors and the nervous system.

The failure of the down-regulation of inflammatory processes as represented by persistently raised levels of pro-inflammatory cytokines such as IL-1β may be responsible for ongoing symptomatology. An elegant prospective study by Gwee et al. harvested rectal mucosal biopsies from PI-IBS patients, a positive control population comprising patients who had contracted gastroenteritis but not the sequelae of PI-IBS, and a negative control population who did not present with gastroenteritis.[187] Flexible sigmoidoscopy was carried out at the index infection and three months afterwards. Reverse trancriptase polymerase chain reaction (rt-PCR) techniques assessed the expression of IL-1β mRNA and its receptor antagonist IL-1ra. The results showed significantly raised IL-1β mRNA in PI-IBS patients compared to the active control at 0 and 3 months, and that this difference was not only persistent over time but seemed to become more accentuated over time. The difference in IL-1β mRNA expression between the positive and negative controls reached statistical significance.

At an immunohistochemical level, an earlier study by Chadwick et al. featured colonic mucosal biopsies of patients who satisfied the Rome 1 criteria for IBS and a group of asymptomatic controls.[188] 38 of the 77 IBS patients had normal histology but increased intra-epithelial lymphocytes and lamina propria CD3+ and CD25+ cells compared to controls. 31 of the 77 patients had non-specific microscopic inflammation on histology and increased numbers of neutrophil lymphocytes and mast cells. The final group, numbering 8 of the 77 patients, had histological and immunohistochemical features consistent with lymphocytic colitis.

Gonsalkorale et al. suggested a genetic imbalance of pro-inflammatory to anti-inflammatory cytokines.[189] Spiller et al. showed an increase in activated T cells, mast cells and pro-inflammatory cytokines in P-IBS patients.[190]

A study by Söderholm et al. used maternal separation rat models to integrate the concept of stress and cytokine dysfunction into the overall understanding of disease pathogenesis. The authors demonstrated increased intestinal permeability and HPA axis activation by stress induced by maternal separation, leading to colonic mucosal barrier dysfunction.[191]

In a subsequent study, the addition of probiotics to manipulate and control the gut microbiota ameliorated the mucosal dysfunction and normalised the release of corticosterone using the same

maternal separation paradigm.[192] The authors hypothesised that stress-induced enhanced mucosal permeability with up-regulation of the HPA axis facilitated the entry of unfavourable bacteria and the propagation of the inflammatory process.[193]

The relationship between cytokine expression and symptom generation is critical to substantiate the role of inflammation in IBS. Work by Barbara et al. highlighted the proximity of mast cells to enteric nerve fibres suggesting a direct interface between the immunological and nervous systems.[194] Furthermore the group presented data that found positive correlations between the density of mast cells and subjective reports of pain intensity.

Animal studies have shown the direct effects of administering cytokines on afferent nerve function. A novel study by Blackshaw's group isolated PBMCs from two separate populations, namely IBS patients and a control sample. The addition of the cytokine-rich PBMC milieu to the mucosal rodent spinal afferent produced a 60% increase in afferent nerve transmission from the supernatant taken from IBS patients but not controls. Whether this is sufficient evidence to explain the differences in visceral perception between the two groups remains to be seen. However when viewed in conjunction with the aforementioned Barbara et al. paper, it offers valuable insights into the potential for direct manipulation of the nervous system through local inflammatory changes.

Other evidence that endorses the role of inflammation on visceral hypersensitivity is the scientific interrogation of the more traditional inflammatory disease states such as Crohn's disease or ulcerative colitis. Some studies have identified increased rectal sensitivity in active Ulcerative Colitis.[195,196] However Chang et al.'s paper found an attenuation of rectal perception in patients with mild chronic Ulcerative Colitis. In contrast the IBS patients in the separate arm of the study were found to have exacerbated rectal perception.[197] This led the authors to conclude that variations in the local inflammatory environment may be responsible for this observation. Conversely, the up – regulation of centrally-driven anti-nociceptive pathways primed by months, even years of chronic inflammation may be the driving mechanism.

Mucosal Permeability

Spiller et al. assessed gut permeability in PI-IBS patients using lactulose/mannitol ratio testing.[190] Lactulose/mannitol permeability studies require a subject to drink an oral load of lactulose and mannitol. Overnight urinary collection allows one to compute the fractional excretion of each sugar- the ratio of total urinary sugar excretion is compared to the total oral dose. Finally the lactulose–

mannitol ratio is calculated as the fractional excretion of lactulose divided by that of mannitol, with normal values being less than 0.025.[198] The Spiller study showed that infection with Campylobacter jejuni enteritis was associated with increased gut permeability during the acute phase and at a 3 months. Furthermore, histological assessment showed raised EC cells and intra-epithelial cells.

Spiller's data set was endorsed by Marshall et al.[199] The Marshall study revisited a population of patients who had dual infection with Campylobacter and E. coli following the widespread contamination of a municipal water source in Walkerton, Ontario. This outbreak affected as many as 2300 local residents leading to twenty-seven patients developing haemolytic uraemic syndrome and six deaths. 132 IBS patients (27 of these patients had the diagnosis of IBS before the outbreak) were compared to 86 non-IBS controls. Lactulose-mannitol ratios were again employed to assess gut permeability. IBS cases were more likely to have increased gut permeability than controls- lactulose/mannitol ratios greater than 0.02 were seen in 35% of the IBS cases compared to 13% of the controls- and increased gut permeability was associated with increased stool frequency in the cases. The interesting point to draw from this is that a significant enteric infection causes a persistently dysfunctional gastrointestinal mucosa, as evidenced by a sustained immunological response and altered permeability. Researchers now hypothesise that altered permeability may serve as an entry point for luminal bacteria and antigens, thereby perpetuating the inflammatory response and maintaining symptomatology.

Dunlop et al. extended the scope of these earlier studies by exploring gut permeability in both the proximal small and the large bowel in PI-IBS and constipation-predominant patients (IBS-C) , and in PI-IBS and non-PI-IBS diarrhoea-predominant patients.[200] The results showed that PI-IBS patients had increased small bowel permeability compared to the IBS-C, and of the diarrhoea-predominant cohort, a non-infectious aetiology appeared to produce a greater increase in permeability compared to the PI-IBS group. What emerges from this study is that enteric infection is an important mechanism in producing sustained mucosal defects, but that other mechanisms are also implicated.

The observation that gut permeability is increased as a result of enteric infection may be due disruption of the tight junctions at the level of the mucosa. A review by Berkes et al. explored the concept of mucosal barrier dysfunction secondary to enteritis from various pathogens including E. coli, Bacteroides fragilis, Campylobacter jejuni and Vibrio cholerae.[201] During an episode of gastroenteritis, the invading pathogen induces a huge inflammatory response, resulting in

alterations in the structure and function of the tight junction barrier, changes in electrolyte and fluid secretion, and stimulation of the inflammatory cascade. In the post-acute phase, the inability to restore the integrity of the mucosal barrier by repairing the tight junction interface may be a pathogenetic feature of PI-IBS.

Research from different disease processes can help to determine whether the features of increased gut permeability have any significant role in the propagation of symptoms. Increased gut permeability has been shown to identify Crohn's patients in clinical remission who have an increased risk of clinical relapse.[202] Increased gut permeability has been noted in first degree relatives of those with Crohn's disease.[203] Other studies investigating Coeliac disease have discovered increased gut permeability, a mechanism that possibly allows the unchecked entry of gluten and other antigens to interface with the lamina propria thereby starting and maintaining an immunological response.[204] A salient paper by Clayburgh et al. uses a rat model of T cell-mediated diarrhoea featuring a myosin light chain kinase knockout species (MLCK).[205] MLCK produces phosphorylation of myosin II light chain (MLC), altering the contractility of the mucosal cytoskeleton and promoting permeability. By knocking out MLCK, the data showed that tight junction disruption, protein leak and diarrhoea induced by T cell activation was inhibited. The relevance of MLC phosphorylation in the clinical area is corroborated by research that shows increased MLC phosphorylation in inflammatory bowel disease.[206] Furthermore, in vivo and in vitro studies have established a link between TNF-α and increased MLC phosphorylation.[205,207]

VISCERAL HYPERSENSITIVITY

Recto-sigmoid distension studies have shown lowered pain thresholds in IBS patients compared to healthy volunteers.[208] Visceral hypersensitivity, colonic dysmotility, infection and immune activation have been implicated in altering pain thresholds at a local gut level in IBS. At a brain level, central processing abnormalities of sensory afferent signalling have been described. Previous work on human visceral pain models, suggests that the final expression of symptoms is determined by gut related processes in conjunction with cortical perception of stimuli. Studies on the cortical processing of visceral afferent information investigated the mechanisms in non cardiac chest pain as a disease model. Cortical evoked potentials (CEP) to oesophageal stimuli revealed three distinct clinical phenotypic profiles.[209] One group described as over-reporters of symptoms showed no increased pain threshold and no changes in CEP waveforms compared to healthy controls. The second group with low pain threshold showed two distinct profiles: one with enhanced cortical input

detected as reduced latency and CEP peak amplitude suggesting true heightened visceral afferent input with normal latency and low CEP amplitude in keeping with a hyper vigilant state. The rectal equivalent of this model has been validated in healthy volunteers.[210]

AUTONOMIC DYSFUNCTION

Murray et al. investigated autonomic function in IBS patients by assessing rectal mucosal blood flow (used as a biomarker for autonomic function) and vesico-somatic sensitivity following acute psychological and physical stress.[211] Physical stress was elicited by the cold pressor test in which a hand is placed in a bucket of iced water as the test is being conducted. He demonstrated that acute stress alters gut-specific efferent autonomic innervation in both controls and patients with IBS, although normalisation is delayed in IBS.

HYPOTHALAMIC-PITUITARY AXIS

The emerging role of the Hypothalamic-Pituitary-Adrenal Axis (HPA axis) in the pathogenesis of IBS has focused attention on the hypothalamic hormone, corticotrophin-releasing factor (CRF). CRF signalling pathways are fundamental components of the acute stress response because they induce endocrine, behavioural, and immune changes in response to the impending danger. Studies investigating CRF1 pathway activation induce clinical symptoms similar to that of diarrhoea-predominant IBS, namely abdominal discomfort, diarrhoea, anxiety, and hypervigilant behaviour. Physiological effects have been studied by intravenous or intraperitoneal injection of CRF agonists, resulting in gastric stasis and increased colonic motility.

HPA axis over-stimulation is associated with the pathophysiological development of stress-related psychiatric co-morbidity. Primary psychiatric disease such as schizophrenia, panic disorders and depression are associated with IBS-like symptoms. Conversely there is an increased prevalence of psychiatric co-morbidity in patients with IBS. The relationship between psychiatric symptomatology and IBS is so profound (and the HPA axis lies at the heart of this) that the administration of selective CRF1 receptor antagonists as a novel treatment for patients with severe depression had the additional effect of improving colonic motility and visceral hypersensitivity in a sub-group of treated patients who had concomitant diarrhoea-predominant IBS.

CHAPTER ONE, SECTION 3:

RESEARCH INSTRUMENTS USED IN THE STUDY PROTOCOLS

CLINICAL SCORING SYSTEMS

IRRITABLE BOWEL SEVERITY SCORING SYSTEM

The Irritable Bowel Severity Scoring System (IBSSS) was developed by Professor Whorwell's group in Manchester to serve as a simple, concise clinical symptom severity scoring tool for IBS.[212] Drossman's Functional Bowel Disorder Severity Index (FBDSI) had been championed 2 years earlier for this purpose.[213] However, it was a global symptom assessment index directed at functional bowel disease, i.e. it was not specific to IBS, and therefore was thought to be too underpowered to characterise the day to day changes in IBS symptom severity.

The IBSSS assesses the various dimensions of the IBS presentation, ranging from primary colonic symptoms, extra-colonic symptoms, quality of life measures and psychological profiling. The questionnaire comprises 5 items that reflect symptoms activity over the last 10 days: (1) severity of abdominal pain, (2) frequency of abdominal pain, (3) severity of abdominal distension or tightness, (4) dissatisfaction with bowel habits, and (5) interference of IBS with life in general. The first page comprises demographic data, the second page features the severity scoring system, and pages three and four explore additional information.

In its trial phase the IBSSS was completed by 61 IBS patients of varying degrees of clinical severity (determined by a preceding clinical consultation, and classified as mild, moderate or severe), and 40 randomly selected controls. The questionnaire data was collected independently by a person blinded to the results of the clinical assessment. An additional group of 40 IBS patients were asked to complete the questionnaire a second time (within 24 hours of their clinical consultation) to assess for reproducibility of the data. A final group completed the questionnaires before and after a trial of hypnotherapy to determine the sensitivity of the questionnaire to clinical change.

Statistical analysis showed that controls scored less than 75 out of a possible total of 500. Mild IBS scored between 75 and 175, moderate IBS scored between 175 to 300 and severe IBS scored greater than 300. IBS patients scoring less than 75 were deemed to be in clinical remission. The questionnaire produced reproducible data, and showed that those patients judged to be clinically

improved after treatment had a statistically significant improvement in their severity scores compared to baseline.

VERBAL DESCRIPTOR VISUAL ANALOGUE SCALE

The Visual Analogue Scale (VAS) is a validated psychometric instrument for assessing a subject's agreement with a statement.[214] The subject is asked to mark a position on a continuous line between two end-points which represent the polar opposites of no agreement and total agreement respectively. Visual analogue scales have been used for many years, and have found particular use in the arena of subjective pain assessment.

Visual analogue scales are quick to perform and calculate data,[215,216] produce continuous *or analogue* data (compared to alternative scoring systems such as the Likert scale which produces discrete scores[217]), facilitate the use of parametric statistical analysis, and can be used regardless of language barriers.[218]

Variations on the theme of visual analogue scales exist, according to the orientation of the scale (vertical or horizontal), the size of the scale, the presence or absence of verbal descriptors, whether the scales are graded or not, or even whether the scales have a curvilinear format. A study by Sriwatanakul et al. concluded that a horizontal linear graded VAS without verbal descriptors was the optimal configuration.[219]

Deficiencies of the VAS include the fact that health professions can identify a 1 point change on a Likert scale far more easily than a 10 point change on a VAS,[216] some patients find the VAS difficult to complete and comprehend,[220] and that if the VAS includes grading, verbal or numerical labels, then it predisposes to a clustering of responses around the labels, making data interpretation more difficult.[221]

The VAS has been used widely in the research of Irritable Bowel Syndrome for the assessment of symptomatology, to determine drug efficacy in therapeutic trials and to serve as an instrument for quality of life measurements.[212,222,223]

PSYCHOMETRIC TESTING

HOSPITAL ANXIETY AND DISEASE SCALE

This is a scale that was proposed by Zigmond and Snaith in 1983 to assess whether anxiety and depression were contributing to the distress of patients who attended non-psychiatric departments in general hospitals.[224] It specifically targeted a group of patients that were physically ill, rather than having suspected primary psychiatric disease. The Hospital Anxiety and Depression Scale (HADS) was built around the concept of anhedonia, a sensitive marker of biological dysfunction secondary to depression. The authors postulated that this state of "biogenic depression" would be drug-responsive. The HADS discarded the all-inclusive definitions of depression that described a much broader range of psychological upset. This was an important distinction since earlier self-assessment questionnaires concentrated on somatic symptomatology, and as a result they were less applicable for the assessment of patients with physical illness.

The HADS was readily embraced owing to the fact the scale was easy to complete, it achieved a consistency of results that stood comparison with data from other more ponderous and unwieldy tests, and because it was amenable to being translated into many different languages. Furthermore, it did not assess severe psychopathological symptoms, making it altogether more palatable for medical rather than psychiatric patients. This meant that it was powered to identify the more subtle presentations of psychiatric disorder. Biological symptoms of depression which could also be explained by concomitant medical illness were omitted. These included symptoms such as headache, weight loss, dizziness and fatigue. In practice, the HADS seemed much better equipped to be the quick and efficient research tool that clinicians demanded.

The Hospital Anxiety and Depression Scale (HADS) has two sub-scales, with seven questions each being allocated to the analysis of anxiety and depression respectively. Each item is scored on a 4 point scale from 0 to 3, allowing for a maximum score of 21 in each category. Zigmond and Snaith originally proposed two thresholds to discriminate cases from non-cases. A score of 8 or more defined the low threshold. A high threshold was denoted as being a score of 11 or more. This was more discriminatory and identified those patients who have a high probability of suffering from the mood disorder.

In the construction of the HADS, Zigmond and Snaith had been influenced by the Klein's hypothesis that anhedonia was a type of depressive disorder that reflected neurotransmitter disturbance, a state likely to resolve spontaneously or be ameliorated by antidepressant therapy. Anhedonia is one of the two criteria required for the definition of "major depressive disorder". Its adoption as the driving force for the depression sub-scale was inspired. It serves as a non-specific marker of depression, allowing the scale to cast aside somatic symptoms.

The HADS is a competent and reliable instrument for isolating possible cases of psychiatric disorder. In clinical practice, someone who scores highly on the HADS would then be subject to a comprehensive psychiatric work-up, in which a definitive diagnosis would be made and appropriate treatment implemented.

EYSENCK PERSONALITY QUESTIONNAIRE

The Eysenck Personality Questionnaire is an evolution of previous work on psychometric and experimental studies that expounded the dimensions of personality. It was first published by Eysenck in 1975, and later revised in 1985. Its lineage dates back to the publication of the Maudsley Medical Questionnaire, an instrument used to assess neuroticism or emotionality (N scale). The dimensions of extraversion and introversion (E scale) were added to this questionnaire to produce the Maudsley Personality Inventory. Later additions included the dimension of lie (L scale), published in the Eysenck Personality Inventory. The final new variable, the dimension of psychoticism (P scale) gave birth to the Eysenck Personality Questionnaire.

Eysenck's work was born out of the concept that personality flowed from one's genetic inheritance. He described this as one's temperament. Temperament is an immutable force- the primary component of personality- and that learned behaviour was of secondary importance. The Dimensions of Personality (Eysenck, 1947) identified two major and contrasting dimensions of personality, Extraversion-Introversion and Neuroticism. This provided a two dimensional landscape in which the previous hypothesis of temperament- dating back to Hippocrates- could be inserted. This defined four temperaments or personality types. These included choleric type (high N and high E), melancholic type (high N and low E), sanguine type (low N and high E), and phlegmatic type (low N and low E). Extraversion was defined as the ability to enjoy positive events, whereas neuroticism was the tendency to experience negative emotions. E and N combine to form the most robust description of personality available.

The Scientific Study of Personality (Eysenck, 1952) suggested that psychoticism was the elusive third dimension of personality and that it was independent of E and N. It was based on the concept that psychopathology was a progression of clinical severity. In other words, psychopathology emanated from exaggerations beyond the norm, rather than being a distinct entity. Therefore psychoticism outlined traits of psychoticism that at their most extreme form would give rise to frank psychosis. Within the normal ranges, they are nothing more than traits. The other construct that formed Eysenck's hypothesis was that psychosis and neuroticism were independent of each other. Heath and Martin (1990) went onto to crystallise Eysenck's definition of psychoticism, "It is conceptualized as a continuum of liability to psychosis (principally schizophrenia and bipolar affective disorder) with 'psychopathy' (i.e., anti-social behaviour) defined as 'a halfway stage towards psychosis'".

The Extrovert

This is a personality trait that describes someone who is sociable, outgoing and who has many friends. He is generally impulsive, likes practical jokes and enjoys change. He finds studying and reading difficult, and can be short-tempered and aggressive. Extroverts are chronically under-aroused- his personality is dependent on external stimulation for cortical arousal, as described in Eysenck's arousal theory of extraversion. Cortical arousal has an optimal level- under or over-arousal lead to deficiencies of performance and productivity.

The Introvert

An introvert is chronically over-aroused. This leads to the desire to seek out peaceful, tranquil environments in compensation. An introvert is reserved and retiring, likes books, is serious about day-to-day affairs and shies away from excitement. He is reliable, avoids displays of aggression, and does not act on impulse.

The Neurotic (Emotionally Unstable)

This is an individual who worries all the time, is moody and anxious and prone to depression. He reacts too quickly to emotional issues and at a level out of proportion to the stimulus. Furthermore it takes much longer for him to return to a normal range of functioning following emotional upset. His personality is characterised by negative affect, namely anxiety and depression.

The Emotionally Stable

These individuals remain composed and in control, displaying good emotional mastery. Emotional response in extreme situations is slow, progressive and quick to return to baseline. In short, these people remain undaunted by pressure situations.

The Psychotic (Tough-Mindedness)

A lonesome figure removed from any care or sympathy for his fellow man, and displaying a readiness for hostility and aggression. He is devoid of feelings and emotional sensitivity, and finds the concept of socialising difficult and awkward. He likes to make fun of and denigrate those around him whether they are strangers, loved ones or family, and there is an undercurrent of cruelty and inhumanity to his social interaction. This is compounded by an unhealthy taste for danger, impulsiveness, non-conformity and a penchant for the eccentric. The polar opposite to psychoticism is socialisation (low P scores).

With the incorporation of the psychoticism scale into the Eysenck Personality Inventory, Eysenck was keen to allay fears that those scoring highly on the P scale had pathological phenotypes- the EPQR was a measure of psychological variables based around a normal non-pathological population, and should therefore only be used in said populations.

The Lie Scale

This is a measure of dissimulation. It describes a tendency to pretend that things are better than they actually are. Eysenck and Michaelis demonstrated that the L scale can be manipulated by situations in which there are greater (or lesser) motivations to dissimulate. They showed that if you place individuals in low dissimulation environments lower L scores would be recorded. Conceptually this would advocate that if there are lower L scores, then the test should become more unreliable and inconsistent, since there should be a greater variability in those factors that the test does not examine. Empirical data does not support this- there is no lowering of the reliability of the test in an environment of low motivation for dissimulation. This would suggest that in addition to dissimulation, the L scale also unearths a stable personality factor which Eysenck proposed as some degree of social naivety or conformity. Another characteristic of the L scale is that scores increase with age. X speculated that this was part of a social desirability model- as people get old and infirm, they contend to others that all is well when the opposite may be so.

Criminality Scale

Combined analysis of the three personality scales across two groups of individuals, criminals and non-criminals, highlighted a number of questions that proved to be highly discriminatory. These questions were incorporated into an offshoot scale, the Criminality scale, that served to distinguish those with criminal, recidivist tendencies from those who did not.

Addiction Scale

Analysis of a cohort of drug addicts compared to controls allowed Eysenck to determine which of the questions in the EPQ were powered to differentiate these two groups. Thirty-two questions were selected, each with a statistical significance of $p < 0.001$ (in the male population) to enable this differentiation. Test-retest reliability analysis in subsequent studies proved that the Addiction scale was robust and reproducible.

Clinical Application of the EPQ-R

The EPQ-R must not be used as a blunt instrument to identify and diagnose psychiatric comorbidity. Its purpose is to examine an individual for subtle psychological traits that may give some insight into the why an individual is behaving in a certain way, and why he or she is finding the challenges of life so formidable.

Eysenck and IBS

PubMed searching using the parameters “Eysenck” and “Irritable Bowel Syndrome” yielded 8 results. Toner et al. found greater L scale scores in IBS patients compared to controls.[225] They proposed a conceptual model asserting that some IBS patients have a self-schema, an ingrained knowledge of self, built around social desirability. Dinan et al. confirmed that IBS patients had greater scores for neuroticism and introversion compared to patients with peptic ulcer disease.[226] Increased neuroticism in female IBS patients but not male IBS patients was noted by Fock et al.[227] who compared IBS with inflammatory bowel disease controls. Fock also showed that there were lower L scores in the IBS group compared to the IBD controls. In contrast, Talley found that IBS was significantly associated with somatisation (using the Psychosomatic Symptom Checklist) and lifetime depression but not neuroticism.[228] Two years later he concluded that neuroticism and psychological co-morbidity were not significant predictors in health-seeking behaviour in community subjects with IBS.[229] Talley once more employed the EPQ to investigate the relationship between abuse and IBS. Data showed that the IBS group had higher neuroticism scores compared to non-IBS responders, and

that abuse in childhood but not adulthood was associated with IBS using univariate analysis. Given that neuroticism and psychological morbidity were also univariately associated with abuse in childhood and abuse in adulthood and IBS, they hypothesised that abuse may induce the expression of neuroticism that precipitates the later development of IBS.[75]

WHITELEY INDEX

The Whiteley Index was developed by Pilowsky in 1967 and is considered to be the definitive instrument for assessing hypochondriacal beliefs and anxieties.[230] Pilowsky asked 100 medical, nursing and ancillary staff of the United Sheffield Hospitals to give their own definitions of hypochondriasis. These definitions were pooled together, and a questionnaire was born from the various observations.

In the 40 years since its publication, the Whiteley Index, as it became known, is still one of the most widely used scales for assessing hypochondriasis.

PATIENT HEALTH QUESTIONNAIRE 15

The Patient Health Questionnaire-15 is a 15 point questionnaire developed from the Patient Health Questionnaire (PHQ). The Patient Health Questionnaire[231] was conceived as a self-administered version of the PRIME-MD diagnostic instrument for common mental disorders.[232]

The PRIME-MD screened for the five most common psychiatric disorders in primary practice, namely depressive, anxiety, alcohol, somatoform and eating disorders. It has been widely used, and its refinement into easy to complete, self-administered versions, namely the PHQ (and the abbreviated PHQ subsets) makes it even more attractive.

The PHQ was published by Spitzer et al. in 1999.[231] Spitzer had concluded that even though the PRIME-MD only took 8.4 minutes to administer, it was still considered to be too time-consuming for primary practice. The PHQ took the 2 central components of the PRIME-MD, the patient questionnaire and the clinical evaluation, and concentrated the process into a 3 page form. It assesses eight diagnoses, divided into *threshold disorders* (which correspond to specific DSM-IV diagnoses: major depressive disorder, panic disorder and bulimia nervosa) and *subthreshold disorders* (disorders whose criteria encompass fewer symptoms than are required for any specific DSM-IV diagnoses). The subthreshold disorders include other depressive disorders, other anxiety

disorders, probable alcohol abuse or dependence, binge eating disorder and probable somatoform disorder).

The PHQ-15 was a further exercise in reductionism.[233] It grouped together the 15 questions in the PHQ that examined for somatisation beliefs. It is therefore an instrument that focuses entirely on this particular disorder. It is limited by the fact that it cannot distinguish between medically explained and unexplained somatic symptoms, and therefore must be used as a measure of somatic symptom severity rather than an instrument to diagnose somatoform disorders. In practical terms, anyone scoring highly on the PHQ-15 should be assessed to see if there is any medical pathology to explain the symptoms.

VISCERAL SENSITIVITY INDEX

The Visceral Sensitivity Index (VSI) is an instrument that was designed to assess GI symptom-specific anxiety (GSA). GSA is though thought to play a role in IBS symptom generation and propagation, and may be considered to be an endogenous stressor.[234] Stressors that affect gut physiology and the brain-gut axis are mediated by the emotional centres which mediate outputs via neuroendocrine, autonomic, pain and attentional pathways. Mayer proposed a stress model that looked at the role of stress in IBS. Stressors have different functions and may occur at different time points in the cycle of symptom generation. Some may cause permanent enhancement of the stress response (pathological stress), some precipitate transient increases in symptom severity, and some cause perpetuation of symptoms (symptom-generated stress). Figure 1.3.1 explains this relationship.

Figure 1.3.1 Role of Stress in the development and modulation of IBS

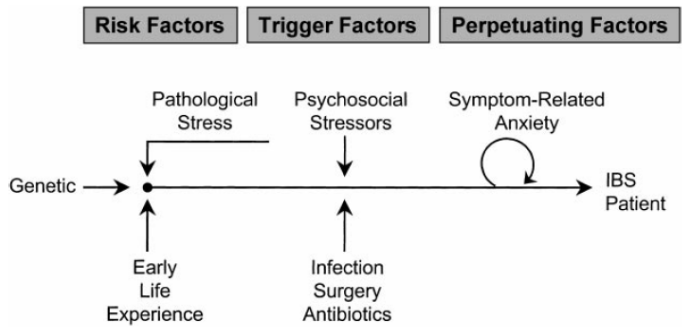


Fig. 1. Role of stress in development and modulation of irritable bowel syndrome (IBS) symptoms. Different types of stressors may play a role in the permanent biasing of stress responsiveness, in transient activation of the stress response, and in the persistence of symptoms.

Adapted from Mayer EA et al. Am J Physiol Gastrointest Liver Physiol, Vol. 280 Issue 4, 2001.[234]

In trying to define GSA, Mayer et al. referred to earlier work by Spielberger and Reiss in terms of the conceptualisation of anxiety sensitivity and state trait anxiety, in addition to their own work on biobehavioural modelling in IBS.[235-237]

They concluded that GSA had five dimensions:

1. Worry: obsessive, negative, catastrophic thoughts, either learned or innate, reflecting a negative, future-oriented perspective on GI symptoms and their impact.
2. Fear: learned responses to previously innocuous physical sensation(s) that have been paired with a painful or aversive stimulus; often unassociated with feelings of anxiety.
3. Vigilance: an increased awareness of, attention to, and increased perceived relevance of GI-specific sensations resulting in a decreased ability to attend to other internal or external stimuli.
4. Sensitivity: heightened perception of GI-specific sensations and symptoms under a variety of conditions including eating and stress.
5. Avoidance: increased safety behaviours, rituals, and seeking reassurance to GI-specific sensations.

The VSI has yet to make a significant impact in the field of IBS, largely due to the fact that it is such a new instrument. However, revalidation of the VSI was performed by Labus et al. confirming that the VSI demonstrated concurrent, divergent and discriminant validity, and appeared to explain the relationship between general psychological distress measures and gastrointestinal symptom severity. Furthermore, the VSI was related solely to gastrointestinal (and not non-gastrointestinal) symptom severity.

SPIELBERGER STATE TRAIT ANXIETY INDEX

Cattell and Scheier proposed the concept of state anxiety and trait anxiety in 1961.[238] However this distinction could not be made without reference to Freud's exposition of neurotic (trait) anxiety and objective anxiety.[239] The mantle of state anxiety and trait anxiety theory was readily taken up by Spielberger et al. in the 1970s with the publication of the State Trait Anxiety Inventory (STAI).[240] This self-report questionnaire underwent various revisions (to improve its discrimination between anxiety and depression, to improve the psychometric potency of the weaker questions and to improve the factor structure of the trait anxiety), culminating in the definitive questionnaire in 1983.[241]

State anxiety is a temporary up-regulation of the autonomic nervous system caused by the perception of impending threat. State anxiety is context-specific i.e. situational, and the degree of anxiety generated is proportional to magnitude of the perceived threat. In contrast, trait anxiety is a measure of an individual's "anxiety proneness", a much more stable entity akin to a characteristic. Spielberger defined the two entities as follows- "State and trait anxiety are analogous in certain respects to kinetic and potential energy. State anxiety, like kinetic energy, refers to a palpable reaction or process taking place at a given time and level of intensity. Trait anxiety, like potential energy, refers to individual differences in reactions. Potential energy refers to differences in the amount of kinetic energy associated with a particular physical object, which may be released if triggered by an appropriate force. Trait anxiety implies differences between people in the disposition to respond to stressful situations with varying amounts of state anxiety. But whether or not people who differ in trait anxiety will show corresponding differences in state anxiety depends on the extent to which each of them perceives a specific situation as psychologically dangerous or threatening, and this is greatly influenced by each individual's past experience."

The STAI is composed of two separate 20 item scales assessing state and trait anxiety independently. Each item is answered according to a 4 point Likert scale. The state scale questions the subject about how they are feeling at that moment whereas the trait scale inquires about how they generally feel.

The STAI has been adopted by thousands of research studies and has acquired substantial evidence for reliability and validity.[242] It is now considered to be the reference standard for assessing state and trait anxiety. Other scales exist but they have not achieved the same level of prominence.

Quality Of Life Scores

Irritable Bowel Syndrome Impact Scale

The Irritable Bowel Syndrome Impact Scale (IBS-IS) was published in 2005 by Longstreth et al.[243] Its creation was fuelled by a desire to have a psychometric instrument that could assess the negative impact that IBS symptoms have on patients. Previous IBS-specific quality of life questionnaires were not powered to do this in a concise, self-administered format.[244-247]

Development of the IBS-IS incorporated a 7 point Likert scale with questions exploring themes of anxiety, psychological distress, sleep disturbance, fatigue, interference with activities of daily living and dietary avoidance. Interestingly, they discarded questions related to sexual functioning since

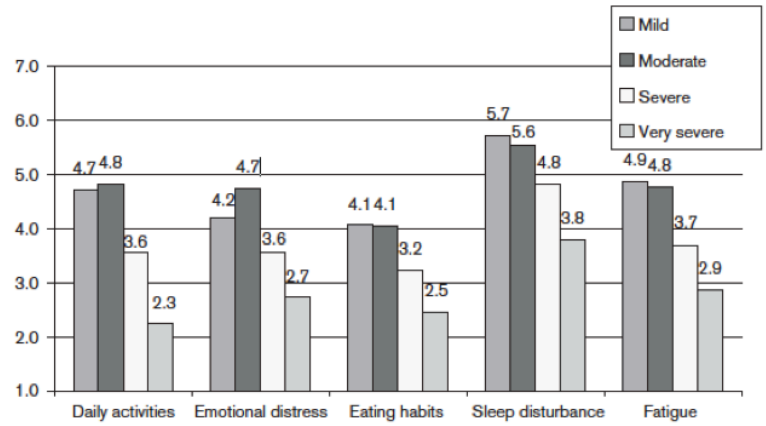
there is traditionally a high non-completion rate for this line of questioning.[248] Other questions involving financial distress and IBS symptoms were discarded. The value of a 7 point Likert scale (rather than a 5 point scale)has been found to be optimal in assessing responsiveness.[249]

The pilot questions devised from literature searches, clinical observation and expert opinion were completed by 155 IBS patients. The subsequent data interpretation removed questions that had a high ceiling affect, questions that appeared to be assessing other psychological factors, and those questions that had a high correlation with other questions (to reduce replication). Factor analysis further distilled the number of questions into its final form, an easy to complete, user-friendly 26 item questionnaire.

Figure 1.3.2 Irritable Bowel Syndrome Impact Scale [243]

416 European Journal of Gastroenterology & Hepatology 2005, Vol 17 No 4

Fig. 1



Irritable Bowel Syndrome Impact Scale (IBS-IS) dimensions and symptom severity.

NEUROPHYSIOLOGY

CORTICAL EVOKED POTENTIALS

In the non-cardiac chest pain model, CEPs differentiated a seemingly homogenous patient population into distinct phenotypic sub-groups according to subjective reporting and CEP waveform analysis.[209] The use of CEPs as an objective assessment of a patient's baseline visceral afferent sensitivity can be harnessed to interrogate sensory afferent dysfunction in FGIDs, particularly in IBS. Using this test, patients can be distinguished by certain neurophysiological parameters according to analysis of the individual components of the cortical response. This raises the possibility that CEPs could facilitate sub-group differentiation of IBS, traditionally seen as a homogeneous entity.

Anatomy

There is a marked contrast in the innervation of the gastrointestinal tract depending on whether the organ has developed embryologically from the foregut or the hindgut. Proximal structures such as the oesophagus or the duodenum have dual innervation. This comes from both vagal and spinal nerve fibres. Structures that are located more distally, for example the sigmoid colon, are innervated by sacral spinal afferents. The types of nerve fibres seen in the visceral system are unmyelinated and without specialized receptor endings. Traditionally these fibres have been sub-grouped according to their degree of mechanosensitivity.

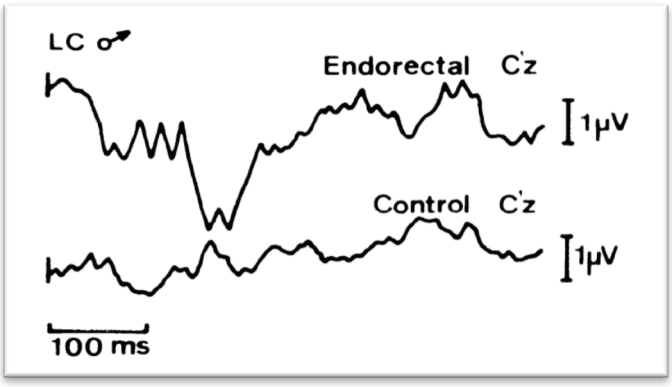
Visceral innervation

Painful stimulation of the viscera results in activation of both spinal and vagal afferents. Whilst vagal afferent activity saturates at innocuous stimulation intensities, spinal afferents encode both innocuous and noxious peripheral activity. The characteristics of the CEP suggest that the cortical response is primarily mediated via spinal afferents, with limited contribution from vagal afferents. Current thinking suggests that vagal afferents have a modulatory effect on pain transmission and this is supported by the fact that vagal afferents project to all of the brain-stem and higher cortical regions that spinal afferents project to. The complexity of this highly integrated system highlights the limitations of studying peripheral nerves in isolation. Visceral pain should be considered to be one part of a complex neurobehavioral system. Cortical Evoked Potentials provide an opportunity to study this system in a degree of detail which was not previously available.

History

Cortical evoked potentials (CEPs) have been used extensively since the 1970s for clinical diagnostic evaluation and represent the sequence of voltage changes generated in the brain in response to an experimental stimulus. A stimulus can use any of the sensory modalities. For example, light may be used to stimulate visual evoked potentials recorded in the occipital cortex. CEPs have been studied in a gastroenterological context since 1987 when Meunier elicited evoked potentials from 3 healthy volunteers in a proof of concept study.[250] Research from the same group the following year published endorectal evoked potentials stimulated by mechanical balloon dilatation (Figure 1.3.3).[251] They demonstrated that the absence of the stimulus using sham testing did not elicit a reproducible cortical response.

Figure 1.3.3 Endo-rectal evoked response



Endo-rectal evoked response from a single subject after 402 distensions of the rectal wall compared to sham testing in the same patient. Adapted from Collet, L et al, Am J Physiol 254:4.

Further research by Frieling in 1989[252] and Loening-Baucke in 1991[253] using electrical stimulation of the rectum defined and categorised the morphology of the evoked potentials, and assessed reproducibility of the traces.

CEP Morphology

The cortical evoked potential can be analysed with regards to the morphology, latency and amplitude of the cortical responses that are precipitated by the peripheral stimulus. The cortical responses take the form of electrical deflections from the baseline level of (cortical) activity. A series of positive and negative deflections from baseline produces the characteristic morphology of the evoked potential.

A peak is the maximum point of deflection on the CEP trace (i.e. the highest or lowest point on the corresponding curve). In the context of electrical rectal stimulation, 5 discernible peaks can be isolated, and are termed as such (Peaks 1 to 5, abbreviated to P1 to P5). With increasing distance from the stimulus the waveforms become less prominent, such that the final peak (Peak 5) can be barely distinguishable.

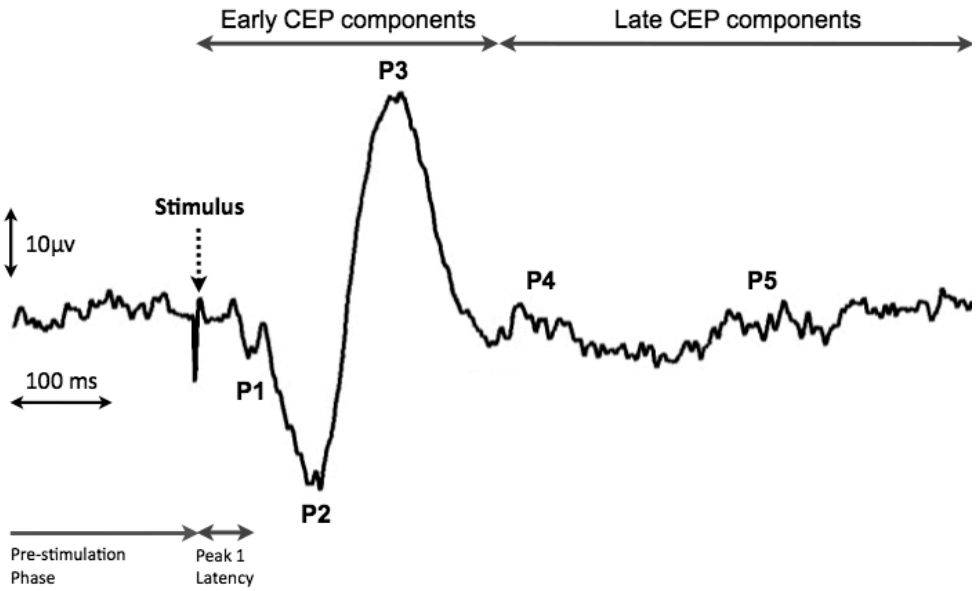
Earlier studies have named the various CEP peaks in terms of positive or negative deflections e.g. P1, N1, P2. This study has changed the nomenclature to make it more accessible. As a result "P" stands for peak rather than positive.

The first part of the cortical response (Peaks 1 to 3) is defined as the early component of cortical activity; Peaks 4 and 5 constitute the late response. The early component, and in particular Peak 1 represents the integrity of the neural circuit that sends sensory information from the periphery to the brain. This involves activation of visceral sensory afferents, the firing of paraspinal sensory nerve pathways and the subsequent arousal of the supraspinal centres. It is at this point that the CEP traces start to appear on the monitor.

Figure 1.3.4 shows a typical evoked potential following rectal electrical stimulation. The peaks are clearly seen. It is therefore a relatively easy process to measure the latency of each peak- this is the time in milliseconds between the stimulus (@ 0 seconds) and the peak. The amplitude of each peak- the height of each peak from baseline- is calculated in microvolts.

Another point to make is that an averaged CEP trace is produced for each cycle of 50 stimulations. If you were to analyse one CEP trace in isolation, there would be so much artefact and noise that the results would be unintelligible.

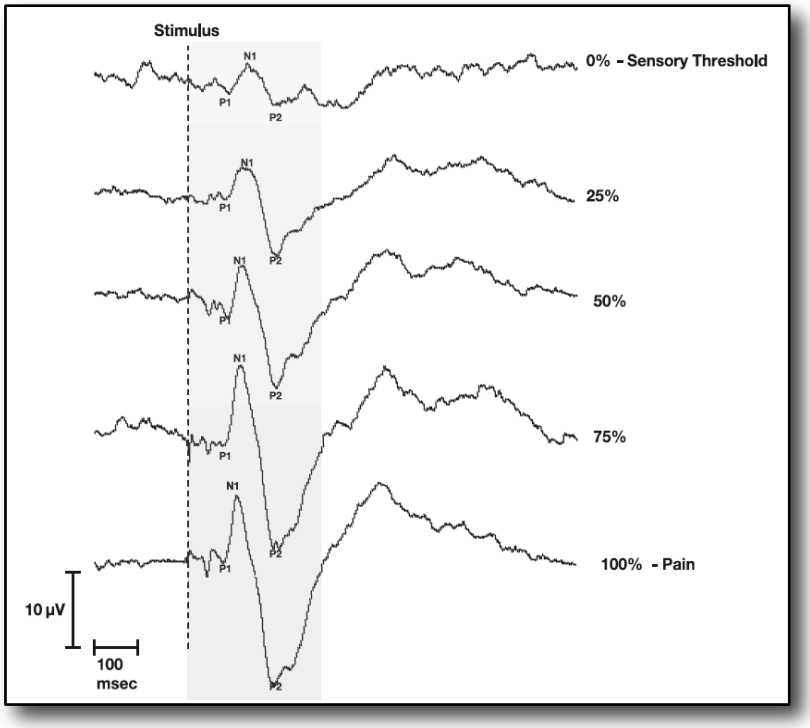
Figure 1.3.4. A Cortical Evoked Potential recorded after electrical rectal stimulation in control subject



Research context of Cortical Evoked Potentials

CEPs can be used to target visceral sensory afferent pathways with sufficient temporal resolution to discriminate between primary and secondary processing of visceral afferent information. In other words, when a rectal electrical stimulus is administered, CEPs can record the primary cortical response to the stimulus followed by a delayed secondary cortical response. This distinction is important because it can distinguish a state consistent with primary visceral hypersensitivity (as defined by decreased latency for a given stimulus) from competing, delayed central contributions influenced by factors such as psychological state and anxiety. Such an approach will indicate whether IBS is predominantly driven by primary visceral hypersensitivity as previous studies have suggested or alternatively by psychological hypersensitivity.

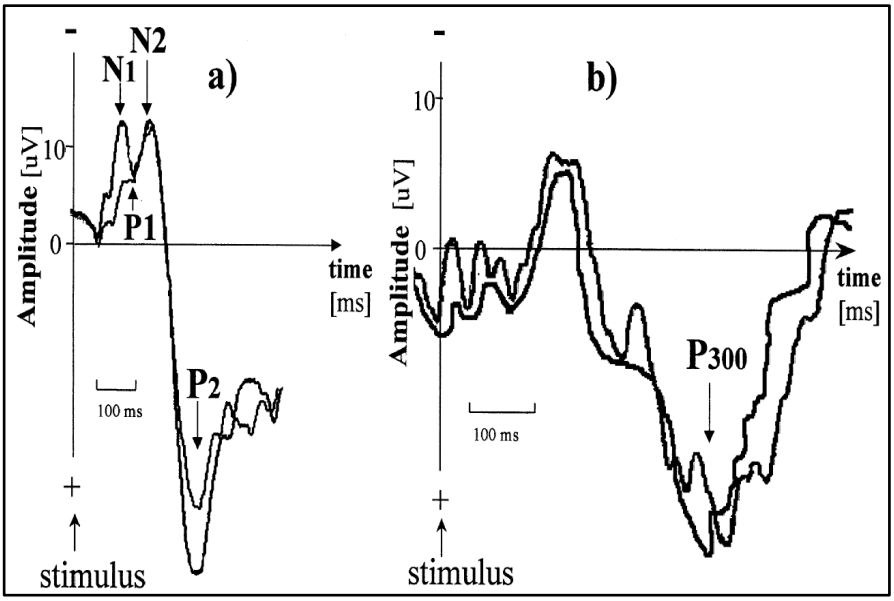
Figure 1.3.5. Rectal evoked potentials using varying stimulus intensities



Adapted from Harris et al. 2006, AJP[210]

Figure 1.3.5 shows rectal CEPs recorded from a female control subject over varying stimulus intensities, graded at 25% intervals between the sensory and pain thresholds. With increasing stimulus, the individual peaks become more prominent, and the peak latencies reduce. The early CEP complex is seen in the shaded area, and represents the first 250ms of cortical activity. The late CEP response represents endogenous brain activity. This late CEP complex is similar to the P300 complexes seen in other studies such as the Hollerbach et al. (Figure 1.3.6).

Figure 1.3.6. Oesophageal evoked potentials during anticipatory stimulation



Adapted from Hollerbach et al, Neurogastroenterology and Motility 1999.[254]

Figure 1.3.6 shows oesophageal CEPs elicited during electrical stimulation of a healthy control. On the left hand side, electrical stimulation produces well-defined, reproducible morphology of the early CEP complex (named using the traditional terminology, the P1, N1 and P2 peaks). On the right hand side, the same subject elicits a cortical response in anticipation of an electrical stimulus i.e. a sham stimulus. What is interesting is that the early CEP complex is lost, but the late complex becomes far more robust. This indicates large scale non-specific cortical activity, as the higher cortical structures prepare for the arrival of the peripheral afferent stream.

In summary, the combination of CEPs with subjective patient reports of discomfort, and quantitative assessment of visceral sensation (as defined by sensory and peak thresholds to electrical stimulus) provides a unique insight into studying the phenomenon of visceral hypersensitivity in IBS.

CHAPTER ONE, SECTION 4:

HYPOTHESIS & AIMS

HYPOTHESIS

Irritable Bowel Syndrome is a heterogeneous population of patients who may be differentiated into discrete sub-populations based on neurophysiological, psychological and immunological evaluation. Defining these groups allows the opportunity to target therapy depending on the underlying pathogenesis.

AIMS

To compare IBS patients with a control population using the following four modalities.

1. Psychological Questionnaires

- To compare psychological profiles of IBS patients with controls. Previous research has demonstrated numerous psychological traits that are more common in IBS, for instance depression. This study is powered by comprehensive psychological questionnaires that can discern dimensions of personality (e.g. neuroticism, introversion, the capacity for lying or criminality), and assess scores for depression, anxiety, hypochondriasis and somatisation.
- To compare the Spielberger State Anxiety Index (SSAI) with the neurophysiological data. The SSAI assesses anxiety at a specific time, and can be used to discern whether a subject's inability to tolerate higher electrical pain thresholds is due to the anxiety of moment (i.e. those anxious but with normal sensation) or due to genuine visceral hypersensitivity (i.e. those relaxed but with genuinely increased rectal sensitivity).

2. Anorectal Sensitivity

- To use electrical stimulation of the rectum to compare sensory function by charting sensory and pain thresholds. Research has previously focused on using recto-sigmoid balloon distension as a surrogate for visceral sensation. This is actually a marker of one's tolerance to balloon distension, since it produces symptoms of impending defaecation rather than actual pain. The continued use of the barostat in such studies is based on the concept that it

is perhaps more physiological than other methods of interrogating visceral sensation in the bowel. Electrical testing may endorse or challenge the utility of the barostat as the gold standard test.

3. Cortical Evoked Potentials

- To document the morphology, amplitude and latency of evoked potentials elicited by rectal electrical stimulation in a control population. Research so far has only described proof of concept studies and suggested possibilities for the clinical application of this test. There has been no attempt to delineate normal ranges, and in so doing, define what is abnormal.
- To assess evoked potentials over 3 separate days of testing in a control population. Reproducibility of data on intra-day or between-day analysis is vital for the validity of a clinical test. This has not been done before in rectal evoked potentials.
- To compare evoked potentials in an IBS population to the control group. Rectal evoked potentials have not been used to assess a disease population. Evoked potentials are being used as a neurophysiological correlate of visceral afferent transmission. Abnormal evoked potentials would suggest altered functioning of this pathway- in the context of expressed pain at low stimulus intensities, a neurophysiological template of visceral sensitivity may become apparent.
- To compare intra-day and between-day evoked potentials in IBS patients. Comparison during a day's testing or over a period of days would identify whether IBS patients become more tolerant of the applied stimulus. This is a process called habituation, where one becomes accustomed to repetitive noxious stimuli. Inability to habituate may be an important mechanism in sustaining visceral hypersensitivity, due to the failure of centrally-mediated pain-inhibitory pathways.

4. Cytokine Analysis

- To analyse cytokine expression in IBS patients and controls. Recent data has proposed a low-grade inflammatory role in the pathogenesis of IBS. This study will use a broad panel of both pro-inflammatory and anti-inflammatory cytokines to try to discriminate the two populations.

CHAPTER TWO:

METHODOLOGY

STUDY DESIGN

Inclusion Criteria

IBS patients were identified by having symptoms consistent with the Rome 2 definition of IBS, and without any known comorbidity that might account for their symptoms.

Exclusion Criteria

Patients with a history of weight loss, psychiatric illness (depression, mania or schizophrenia), active infection, recent antibiotics, anti-depressants or anti-inflammatory medication were excluded.

Study Pathway

Each study participant was subjected to three non-consecutive days of psychological and neurophysiological assessment. The three days of testing were concluded within a 3 month time-scale.

Each subject underwent rectal sensitivity testing to determine sensory and pain thresholds to rectal electrical stimulation. Cortical evoked potentials were recorded following the same electrical stimulus. Psychological questionnaires were completed on each day.

Female patients were tested during the follicular phase of the menstrual cycle to avoid heightened sensory perception during the menses.

At a later stage IBS patients and healthy volunteers were invited to return to the study in order to give blood samples in order to facilitate the cytokine analysis part of the protocol. These subjects underwent further clinical and psychological assessment in addition to having a 20ml sample of venous blood.

Patient Recruitment

Patients with IBS were sourced from outpatient clinics at St. Marks Hospital and Northwick Park Hospital. Healthy volunteers were recruited through local advertisement at St. Mark's Hospital.

Some of the healthy controls worked for the Northwick Park PCT, although they had not previously been involved as participants in other research trials.

Ethics

Ethical approval was granted following a successful application to the Brent and Harrow Research Ethics Committee.

ENDPOINTS

1. Assessment of Psychological Factors

Endpoints were achieved with reference to the psychological questionnaires that were completed. These included Spielberger State-Trait Anxiety Inventory for Adults, Hospital Anxiety and Depression scale, Eysenck Personality Questionnaire- Revised, Visceral Sensitivity Index, Patient Health Questionnaire, Whiteley Index and Irritable Bowel Syndrome Impact Scale.

2. Assessment of Clinical Severity

The Visual Descriptor Visual Analogue Scale documented subjective accounts of the unpleasantness and intensity of symptoms. The Irritable Bowel Severity Scoring System assessed the severity of IBS symptoms.

3. Sensory and pain thresholds

Rectal sensory and pain thresholds were completed on each day of testing.

4. CEP Analysis

Analysis of the CEP waveforms assessed the morphology, amplitude and latency of the individual components of the cortical response.

5. Immunological and Endocrine Endpoints

Peripheral blood mononuclear cells were stimulated with E. coli LPS (serving as a TLR4 ligand) to investigate the stimulated (and non-stimulated) expression of cytokines.

Patient Flow Chart

Outpatients Clinic

Rome 2 Compliance for Irritable Bowel Syndrome

No exclusion criteria

Patient Information Sheet

Day 1

Consent

Questionnaires

Spielberger State-Trait Anxiety Inventory for Adults

Hospital Anxiety and Depression scale

Eysenck Personality Questionnaire- Revised

Visceral Sensitivity Index

Patient Health Questionnaire

Visual Descriptor Visual Analogue Scale

Whiteley Index

Sensory and Pain Thresholds (ST/PT)

Cortical Evoked Action Potentials

Days 2 & 3

Questionnaires

Spielberger State-Trait Anxiety Inventory for Adults

Hospital anxiety and Depression scale

Visual Descriptor Visual Analogue Scale

Sensory and Pain Thresholds (ST/PT)

Cortical Evoked Action Potentials

Blood Test

Psychological Assessment

The questionnaires that were used included the Spielberger State-Trait Anxiety Inventory for Adults (SSTAI), Hospital Anxiety and Depression Scale (HAD), Eysenck Personality Questionnaire-Revised (EPQ-R), Visceral Sensitivity Index (VSI), Patient Health Questionnaire (PHQ-15), Visual Descriptor Visual Analogue Scale (VDVAS), Whiteley Index (WI) and Irritable Bowel Syndrome Impact Scale (IBS-IS).

The SSTAI, HAD and PHQ-15 were performed on each day of testing. In addition, the EPQ-R, VSI and WI were completed on the first day of testing, but not repeated.

Those subjects who enrolled for cytokine analysis completed the IBS-IS, IBSSS, SSTAI, HAD and the VDVAS.

CORTICAL EVOKED POTENTIAL PROTOCOL

Rectal Sensitivity Testing

Subject Preparation

Subjects were fasted overnight and encouraged to defaecate prior to starting the study. Bowel preparation was not be used, thereby preventing aperient-induced anorectal sensitisation. Subjects were positioned in the left lateral position with knees and hips flexed to facilitate the placement of the rectal catheter. After its insertion, subjects were then asked to adopt the supine position.

Rectal Catheter

The rectal catheter was a 3mm nylon tube interwoven with stainless steel braid and sheathed in silicone rubber (Gaeltec, Dunvegan, Isle of Skye IV55 8GU, Scotland, UK.). It featured two 2mm bipolar ring electrodes sited 1cm apart from each other, and 5cm from the distal end of the catheter. The catheter was inserted into the rectum so that the bipolar ring electrodes were positioned 10 cm proximal to the anal verge. The catheter was connected to a constant current stimulator (model DS7A, Digitimer Ltd, UK) which was itself controlled by a trigger generator (model DG2, Digitimer Ltd, UK). The firing of the trigger generator and subsequent discharge of the electrical stimulus from the constant current stimulator was delivered at a frequency of 0.5 Hz using square wave pulses of 500μs duration. The intensity of the stimulus was manually adjusted between 0 and 100mA.

Visual Analogue Scale

Subjects were asked to subjectively grade the intensity of the stimulus using a visual analogue scale (VAS) scored between 1 and 6. Word anchors comprised “being unaware of the stimulus” (1/6), or noticing a “slight sensation” (2/6), “definite sensation” (3/6), slight discomfort (4/6), “uncomfortable” sensation (5/6) or a “painful” sensation(6/6).

Sensory and Pain Thresholds

At the start of each day’s testing, the sensory threshold (ST) and pain thresholds (PT) were determined. The electrical testing begins at 0mA (no stimulus). The current is then progressively increased in 2mA increments until the subject first acknowledges the electrical stimulus (defined as the sensory threshold or 2/6 on the VAS). The stimulus intensity continues to be increased, and the subject is encouraged to communicate their perception of the increasing stimulus using the defined stages of the VAS. At the point at which the perceived stimulus is felt to have transitioned from

being an uncomfortable sensation to a painful sensation (defined as the pain threshold or 6/6 on the VAS), the electrical current is terminated. This cycle of ascending electrical stimulation is repeated two more times, allowing one to calculate average sensory and pain thresholds over 3 cycles.

Preliminary testing to determine sensory and pain thresholds facilitates the calculation of a stimulus intensity appropriate for recording CEPs. Hobday et al.[255] used a CEP stimulus intensity equivalent to the sensory threshold plus 75% of the difference between the sensory and pain thresholds. For example, if the sensory threshold is 20mA and the pain threshold is 80mA, then the test stimulation intensity is 65 mA. In practice, this equates to a subjective perception of a "slight discomfort" or 4/6 on the VAS. This methodology allows comparison between subjects because, even though there may be subtle differences in the sensory and pain thresholds between individuals, the stimulus intensity is increased by the same factor.

Cortical Evoked Potential Testing

Electrode Placement

Silver-silver chloride surface electrodes were placed on the vertex (active electrode), ear lobe (reference electrode) and neck (ground electrode) according to the International 10–20 System of Electroencephalograph (EEG) electrode placement. They were affixed using electrode gel or paste (Elefix, Nihon Kohden, Japan) after the underlying skin had been gently cleaned using alcohol wipes and prepared with a mild skin abrasive (Omniprep, Weaver & Aurora). Scalp impedance was maintained below 5kΩ.

Cortical Evoked Potential Harvesting

During the recording of CEPs the subjects were encouraged to lie still and silent on the examination couch whilst fixing their gaze on a stationary object, thereby minimising motor artefact. The rectal stimulus was delivered at the calculated testing intensity over 4 cycles of 50 electrical stimulations spaced at 5 second intervals. A 5 minute pause interspersed each cycle. Each cycle of 50 stimulations was then averaged to enhance signal clarity. The patient was given 5 minutes to relax after each cycle, and then the testing would continue. Previous work has shown this approach achieved the best signal-to-noise ratio and optimal clarity of the CEP components. At the conclusion of each day of testing, 4 averaged CEP waveforms were calculated.

Cortical Evoked Potential Acquisition

Data was acquired using a CED 1902 programmable signal conditioner (Cambridge Electronics Design). Display and analysis utilised the SIGAVG program v. 6.04 and Signal for Windows v. 3.08 (Cambridge Electronic Design). Amplifier gain was set at 1000 and the recording sensitivity was usually between 25 and 100 μV. The band pass filter settings were 1–100 Hz, and a 50-Hz noise reduction unit was utilised, if needed, to reduce interference from the main electrical supply (Humbug Noise Eliminator, Digitimer Ltd, 37 Hydeway, Welwyn Garden City, Hertfordshire, AL7 3BE, UK). The sampling rate was 4 KHz, and the recording epoch was 1.5 seconds in duration. The first 200 ms of the recording epoch was pre-stimulation time. Each individual epoch was saved, and the average of the run can be viewed during acquisition. An artefact rejection facility was employed to prevent contamination from eye blinks and swallows as well as visual inspection of each sweep.

Evoked Action Potential Analysis

Rectal CEPs were analysed according to morphology, latency and amplitude of the CEP waveforms. CEPs were averaged after each cycle and then compared to other cycles on the same day of testing (intra-day analysis) and on other days of testing (inter-day analysis).

CYTOKINE PROTOCOL

Isolation and Stimulation of Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells (PBMCs) were prepared from blood samples taken from healthy volunteers and IBS patients. 20ml of venous blood was taken and collected in standard heparinised blood bottles. Preliminary testing showed that even short durations of cooling in a refrigerator caused cell lysis and destruction, rendering the blood samples useless. As a result the blood samples were maintained at room temperature and not subjected to a cooling process beforehand. The blood samples were processed within 2 hours of collection.

Materials and Equipment

- 20 ml heparinised whole blood samples
- Dulbeco's Phosphate Buffered Saline (PBS), Ca^{++} and Mg^{++}-free, sterile (Invitrogen)
- Ficoll-Paque Plus (GE Healthcare), sterile
- RPMI (Roswell Park Memorial Institute) Solution combined with Foetal Calf Serum (FCS), L-glutamine and Penicillin/Streptomycin.
- Standard heparinised blood tubes
- 50 ml Accuspin™ centrifuge tubes (Sigma-Aldrich)
- Sterile pipettes
- Centrifuge
- Incubator
- Haemocytometer and cell counter

Isolation of PBMCs

1. Whole blood samples were diluted 1 to 1 with PBS.
2. 20ml whole blood/PBS solution was layered over 20ml of Ficoll-Paque Plus in a 50ml centrifuge tube, taking care that the blood/Ficoll-Paque interface was not disturbed and that there was a clear demarcation between the two substrates.
3. The blood sample/Ficoll was centrifuged at room temperature for 20 minutes. The centrifuge was set at 1500rpm with the brake off.
4. The buffycoat formed at the interface between the plasma and the erythrocytes was extracted and transferred to new centrifuge tubes.

5. The buffycoat was re-diluted 1 to 1 with PBS solution and then centrifuged for 15 minutes. The centrifuge was set at 1100rpm with the brake off.
6. At this point a pellet of PBMCs has formed at the bottom of the tube. The overlying supernatant was gently extracted and disposed of, taking care not to disturb the pellet.
7. The PBMC pellet was re-suspended in 2ml of RPMI.

Cell Counting and PBMC dilution

1. A counting chamber or haemocytometer was used to establish the density of PBMCs in a fixed 10µl sample. A standard cell counter was used.
2. The PBMC solution was diluted with RPMI to ensure a final dilution of $1x10^6$ cells per ml of RPMI.
3. The PBMC solution was then transferred to standard sterile 96 well plates and maintained at 37 degrees Celsius in an incubator, pending the stimulation phase of the experiment.

LPS Stimulation and Incubation

1. 96 well plates were inoculated with E. coli LPS.
2. The plates were incubated for 24 hours, and then frozen in a -20°C freezer until the assays were ready to be analysed.

Cytokine Assay

96 Well Plate Setup

Each well of a 96 well sterile culture plate can hold 250µl of fluid. For this experiment, a total volume of 100µl of PBMCs and E. coli LPS were used in order to make the concentrations easier to calculate.

Each well was dispensed with: 90µl of PBMCs ($1x10^6$ cells per ml concentration)

10µl of E. coli

Materials and Equipment

- PBMCs in a concentration of $1x10^6$ cells per ml of RPMI
- Ultra-pure E. coli 0111:B4 LPS (Invivogen, Catalogue number: tlrl-pelps, 5mg)
- Sterile 96 well tissue culture plates
- Sterile pipettes

LPS preparation from Stock Powders

Concentrations required for the experiments: 0, 0.1, 0.3, 1, 3, 10, 30, 100 ng/ml

1. 5mg dry powder was mixed with 5ml of sterile endotoxin-free water and homogenised to make a solution of 1mg/ml.
2. Serial dilutions were performed as follows:
3. Add 6µl LPS (1mg/ml) to 594µl of RPMI= 10µg/ml (600µl total)
4. Add 20µl of 10µg/ml solution to 180µl of RPMI= 1µg/ml (200µl "stock solution")
5. Add 30µl of 1µg/ml stock to 70µl of RPMI = 300ng/ml
6. Add 20µl of 300ng/ml solution to 180µl of RPMI = 30ng/ml
7. Add 20µl of 30ng/ml solution to 180µl of RPMI = 3ng/ml
8. Add 20µl of 1µg/ml stock to 180µl of RPMI = 100ng/ml
9. Add 20µl of 100ng/ml solution to 180µl of RPMI = 10ng/ml
10. Add 20µl of 10ng/ml solution to 180µl of RPMI = 1ng/ml

These concentrations take into account a further 1 in 10 dilution when added to the RPMI/PBMC solution in the wells.

Cytokine Analysis

MSD Multiplex Technology

The MSD Pro-inflammatory 7-Plex (MS6000 7 Spot Plate Catalogue Number: N71008B-1 from MesoScaleDiscovery, www.meso-scale.com) was used for analysis of the pro-inflammatory cytokines.

The 7-Plex in its title refers to the patented multiplex technology. This means that multiple cytokines can be read from a single well. The multiplex is a sandwich immunoassay- 7 different cytokine autoantibodies are arranged in a discrete pattern of 7 domains per well. Each cytokine has its own individual domain. Therefore in a 96 well plate, there will be 96 wells each producing data from 7 different cytokines.

The technology is an elaboration on the science of immunofluorescence. Mesoscale refer to the technology as electrochemiluminescence detection. However, the principle is the same. The MSD plates are pre-coated with the 7 cytokine auto-antibodies. The cytokine binds to its capture antibody. A labelled cytokine-specific detection antibody then binds to the cytokine. Electrochemical

stimulation causes light to be emitted from the labelled detection antibody which is then read by the analysing machine and quantified into cytokine concentrations.

The 7-Plex MSD plates analyse these pro-inflammatory cytokines: IL-1B, IL-6, IL-8, IL-10, IL-12p70 and TNF-α. An anti-inflammatory cytokine plex array was sourced from the same company to investigate the expression of IFN-γ, IL-2, IL-4, IL-5 and IL-13.

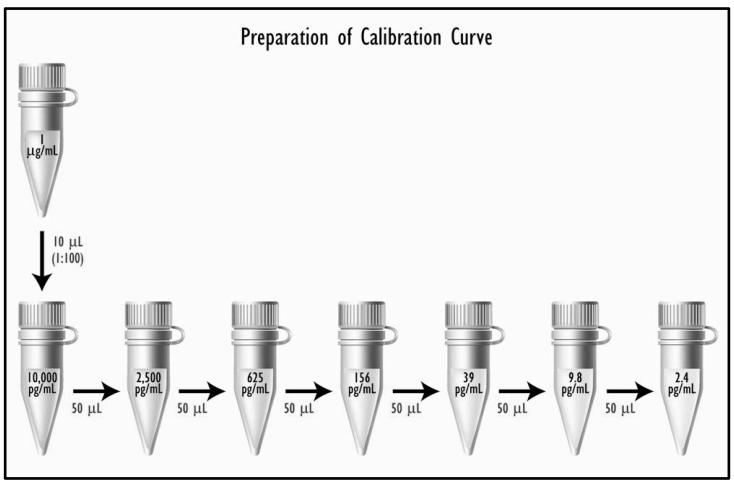
Reagent Preparation

Calibrators were diluted using Calibrator Diluent (CD). The calibration curve preparation outlined below (Figure 2.1) generates a standard curve from 10,000 pg/ml to 2.4 pg/ml. The Calibrator Diluent is a standard tissue culture growth medium with 10% serum.

Preparation of the Calibration Curve

- 10,000 pg/ml: Add 10 µl of the 1 µg/ml stock solution to 990 µL of CD. This high calibrator (10,000 pg/ml) was used to prepare the standard curve following a 1:4 dilution series (as shown below).
- 2500 pg/ml: Add 50 µl of 10,000 pg/ml combined high calibrator (10,000 pg/ml) to 150 µl of CD.
- 625 pg/ml: Add 50 µl of 2500 pg/ml calibrator to 150 µl of CD.
- 156 pg/ml: Add 50 µl of 625 pg/ml calibrator to 150 µl of CD.
- 39 pg/ml: Add 50 µl of 156 pg/ml calibrator to 150 µl of CD.
- 9.8 pg/ml: Add 50 µl of 39 pg/ml calibrator to 150 µl of CD.
- 2.4 pg/ml: Add 50 µl of 9.8 pg/ml calibrator to 150 µl of CD.
- 0 pg/ml: 150 µl of CD

Figure 2.1 Preparation of the calibration curve



Preparation of the Detection Antibody Solution

Detection antibody solutions were kept refrigerated and in the dark to prevent possible degradation by light. The detection antibodies were premixed in solution at a 50X concentration. The working detection antibody solution was diluted down to 1X (or 1.0 µg/ml). For each plate used, 60 µl aliquot of the stock Detection Antibody Mix was diluted with 2.94 ml of Antibody Diluent to achieve the x1 dilution required for testing.

Preparation of the Read Buffer

The Read Buffer T (at a x4 concentration) was diluted 1 in 2 in deionized water to make a final concentration of 2X Read Buffer T. This was accomplished by adding 10 ml of stock Read Buffer T (4X) to 10 ml of deionized water for each plate.

Preparation of the MSD plate

The MSD plate was removed from the refrigerator and allowed to reach room temperature before testing commenced. A blocking step was not employed in this protocol.

MSD Plate Setup

The MSD plate is a 96 well plate. Sixteen of the wells were allocated for the calibration curve in a manner that afforded two separate readings for each concentration of calibrator used. At the point of analysis, these two signals were averaged by the Sector Imager to generate the calibration curve. This is then used as the reference for the rest of the wells (which contained the patient/healthy volunteer samples). This meant that each 96 well plate could test 80 samples. Owing to cost and time implications, there was no redundancy in the samples (i.e. there was no duplication of the samples). This meant that if one well produced unusual results there was not a corresponding duplicate to compare it to.

Figure 2.2 Ninety-six well plate setup for MSD-Plex Cytokine Array

	1	2	3	4	5	6	7	8	9	10	11	12
A	10000 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
B	2500 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
C	625 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
D	156 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
E	39 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
F	9.8 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
G	2.4 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
H	0 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample

Addition of the Sample or Calibrator

25 µL of each Calibrator or Sample Solution was dispensed into a separate well of the MSD plate using reverse pipetting.

Reverse Pipetting

This is a technique designed to minimise the amount of bubbles that are transferred from the sample source into the 96 well plate. A pipette has 2 plunger settings, the first allowing aspiration of a specific volume of sample, and the second deeper setting that expels residual liquid. Reverse pipetting requires you to push the pipet plunger past the first position to the second position prior to aspirating liquid into the tip, thereby aspirating slightly more liquid than the desired volume (overdraw). In order to dispense the liquid from the tip, the pipet plunger is pushed to the first position only. Reverse pipetting results in a much more controlled delivery of the sample into the 96 well plate, minimising bubbles, and therefore maximising the signal.

Incubation of the MSD plate

The MSD plate was sealed with an adhesive plate seal and allowed to incubate for 2 hours with vigorous shaking (300-1000 rpm) at room temperature. This was achieved by using an automated shaking machine. It results in better mixing and more rapid binding of the sample to the capture antibodies.

Addition of the Detection Antibody Solution

25 µL of the 1X Detection Antibody Solution was pipetted into each well of the MSD plate.

Incubation of the MSD Plate

The MSD plate was resealed and then incubated for a further 2 hours with vigorous shaking (300-1000 rpm) using the automated machine.

Washing the MSD plate

The MSD plate was washed three times using an automated plate washer that was fed phosphate buffered saline + 0.05% Tween-20.

MSD plate reading

150 µL of 2X Read Buffer T was dispensed into each well of the MSD plate. Analysis of the MSD plate was then performed immediately afterwards using the Sector Imager.

Analysis using the Sector Imager 6000

The Sector Imager (MesoScaleDiscovery, www.meso-scale.com) was configured to convert the 1 in 2 dilutions of the PBMC assays into 1 in 1 dilutions. In other words, it doubled the analysed cytokine signal to account for the fact that the PBMC assays had been diluted down by a factor of 2 using RPMI. The data was exported into pdf and Excel formats, and then manipulated into the patient database.

CHAPTER THREE, SECTION ONE:

CEP STUDY RESULTS

The results are presented under two major headings- data from the cortical evoked potential analysis (termed CEP Study), and data from the cytokine analysis (termed Cytokine Study). Each of these distinct studies is further sub-divided into demographic, psychological, neurophysiological and immunological sections.

Conventions

Graphs are presented using a hybrid scattergram/box and whisker plot i.e. the same data is represented in both forms side by side. The box and whisker plot is defined according to Tukey's original specification (Tukey, J. W. "Box-and-Whisker Plots." *Exploratory Data Analysis.* Reading, MA: Addison-Wesley, pp. 39-43, 1977). Statistical significance is defined as a p value < 0.05.

Patient Recruitment

Cortical evoked potentials were harvested from 33 IBS patients and 21 controls. All CEP testing was completed within a 3 month timeframe.

Table 3.1.1a Study Demographics

	Controls	**IBS**
Sex	7 Males, 14 Females	6 Males, 27 Females
Mean age	31.4	40.1
Median age	30	38
Mode age	30	30
Age range	21 to 46	20 to 63

Controls were recruited from local advertisement at St. Marks and Northwick Park hospitals, and were healthy volunteers i.e. disease-free. The IBS population were recruited contemporaneously from the outpatient clinics of the very same hospitals. They were recruited consecutively (i.e. in a non-randomised fashion) from secondary and tertiary referrals to these clinics, and serve as a representative sample of this kind (or severity) of IBS patient.

IBS Subtypes

The clinical subtypes of the 33 IBS patients comprised 8 mixed-type, 6 constipation-predominant, 16 diarrhoea-predominant and 3 unsubtyped IBS patients. This is seen graphically on Figure 3.1.1. Diarrhoea-predominance was the most common IBS subtype in our sample. This is contrary to some population-based studies which suggest that the IBS-Mixed subtype is more prevalent.

Figure 3.1.1 IBS subtypes for the CEP study

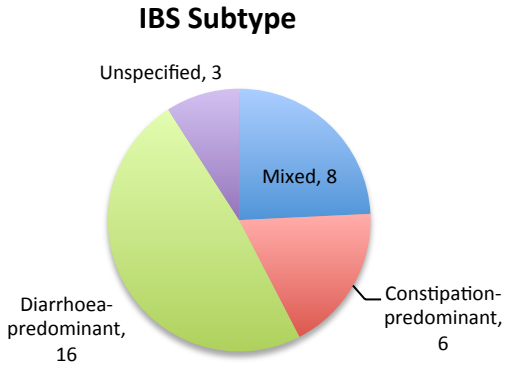


Table 3.1.1b CEP Study Completion

	Controls	IBS
Day 1	21	33
Day 2	19	31
Day 3	19	29

All IBS patients and Control subjects completed the first day of CEP testing. Over the next two days of testing there was some diminution in numbers, but not as much as one might have expected given the nature of the testing. One IBS patient was lost to follow-up after the 1st day; corruption of the CEP data removed a second IBS patient from further CEP analysis on days 2 and 3; a third IBS patient was lost to follow-up after the 2nd day; finally, the 4th IBS patient did not complete the last day of testing since she was due to undergo cosmetic surgery, and fell out of the 3 month window of testing.

Of the 2 control subjects who did not complete the 3 days of testing, the first became pregnant and had to withdraw from the study; the second found it increasing difficult to fit the testing into her schedule, and reluctantly had to withdraw.

It is important to note that there were no withdrawals from the study on account of the invasiveness or discomfort of the CEP testing. Both groups quickly became comfortable with the testing protocol, evident even during the 1st day of testing. This appeared to validate the testing protocol in a practical sense- the protocol was not perceived as being too traumatic.

Study Power

As this was an exploratory study, we arrived at a sample number by considering the results of the Hobson et al. non-cardiac chest pain (NCCP) disease model.[209] This employed a sample size of 29 NNCP patients, and had sufficient power to define clinical subtypes. The data from this study for the discrimination of sensory and pain thresholds between healthy controls and patients with non-cardiac chest pain is tabulated in Table 3.1.1c.

Table 3.1.1c Sensory Thresholds and Pain Thresholds to Oesophageal Stimulation

	Controls	NCCP	*P* value
ST	20.3 ± 5.1	24.8 ± 8.6	.09
PT	72.1 ± 19.4	54.1 ± 23.6	.02

NOTE. All values are shown in milliamperes ± 1 SD.

Post-priori analysis of the pain threshold (PT) data computes a required sample size of 21, a power of 64% and a minimal detectable difference of 15.17mA.

Statistics

One way ANOVA was used to examine for inter- and intra- group differences between the 3 days and repeated measures ANOVA assessed the repeatability of observations between separate days. The mean 3 day difference between IBS and control groups was analysed by the Mann-Whitney test.

PSYCHOLOGICAL ANALYSIS

Psychological analysis was performed using a variety of psychological instruments. These were performed before the anorectal physiology and CEP harvesting. Each study volunteer was left alone in a quiet room to allow sufficient time to reflect and answer the questionnaires.

Figure 3.1.2 Eysenck Personality Questionnaire- Revised: Psychoticism

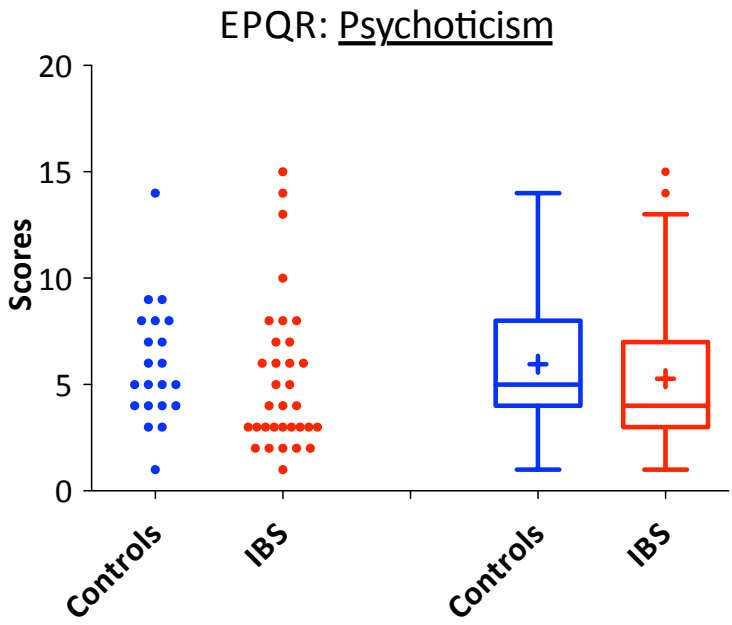


Table 3.1.2 Eysenck Personality Questionnaire- Revised: Psychoticism

Unpaired t test	
P value	0.4636
Mean ± SEM of Controls	5.952 ± 0.6152 N=21
Mean ± SEM of IBS	5.273 ± 0.6205 N=33
Difference between means	0.6797 ± 0.9205
95% confidence interval	-1.169 to 2.528

Figure 3.1.3 Eysenck Personality Questionnaire- Revised: Extraversion

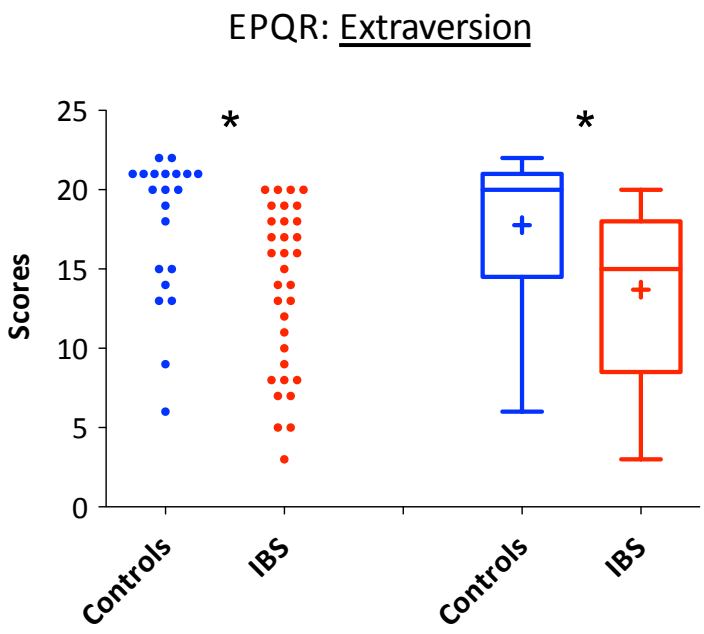


Table 3.1.3 Eysenck Personality Questionnaire- Revised: Extraversion

Unpaired t test	
P value	0.0047
Mean ± SEM of Controls	17.76 ± 0.9950 N=21
Mean ± SEM of IBS	13.70 ± 0.8966 N=33
Difference between means	4.065 ± 1.377
95% confidence interval	1.300 to 6.830

Figure 3.1.4 Eysenck Personality Questionnaire- Revised: Neuroticism

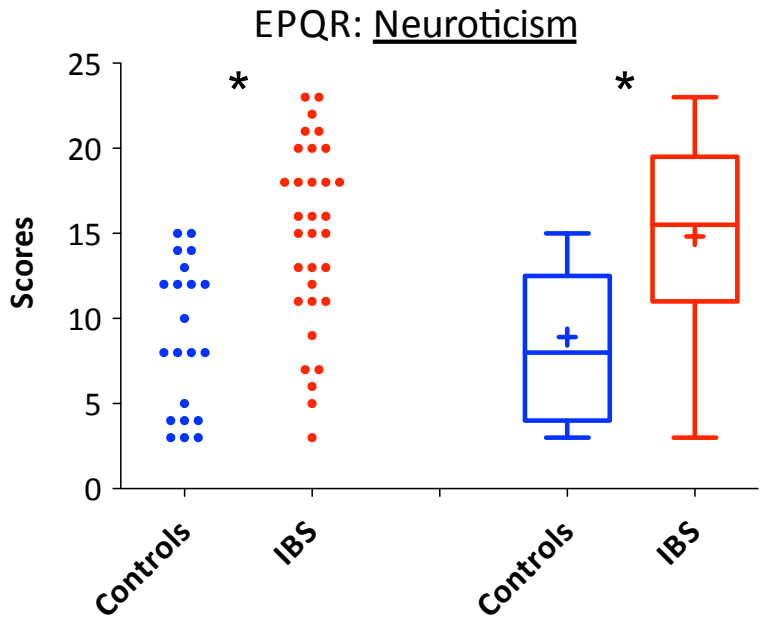


Table 3.1.4 Eysenck Personality Questionnaire- Revised: Neuroticism

Unpaired t test	
P value	0.0001
Mean ± SEM of Controls	8.905 ± 0.9484 N=21
Mean ± SEM of IBS	14.81 ± 0.9677 N=32
Difference between means	-5.908 ± 1.422
95% confidence interval	-8.764 to -3.051

Figure 3.1.5 Eysenck Personality Questionnaire- Revised: Lie

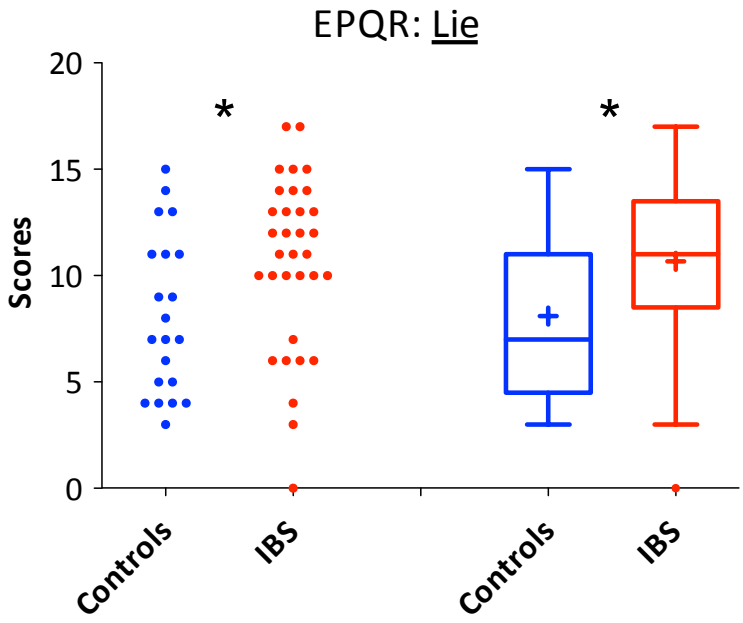


Table 3.1.5 Eysenck Personality Questionnaire- Revised: Lie

Unpaired t test	
P value	0.0225
Mean ± SEM of Controls	8.095 ± 0.8133 N=21
Mean ± SEM of IBS	10.67 ± 0.7013 N=33
Difference between means	-2.571 ± 1.093
95% confidence interval	-4.766 to -0.3764

Figure 3.1.6 Eysenck Personality Questionnaire- Revised: Addiction

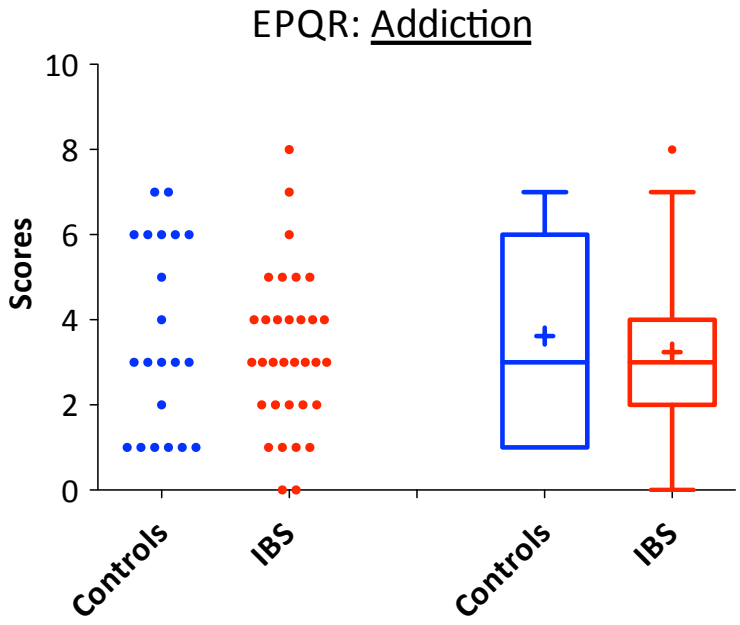


Table 3.1.6 Eysenck Personality Questionnaire- Revised: Addiction

Unpaired t test	
P value	0.5039
Mean ± SEM of Controls	3.619 ± 0.4854 N=21
Mean ± SEM of IBS	3.242 ± 0.3228 N=33
Difference between means	0.3766 ± 0.5596
95% confidence interval	-0.7474 to 1.501

Figure 3.1.7 Eysenck Personality Questionnaire- Revised: Criminality

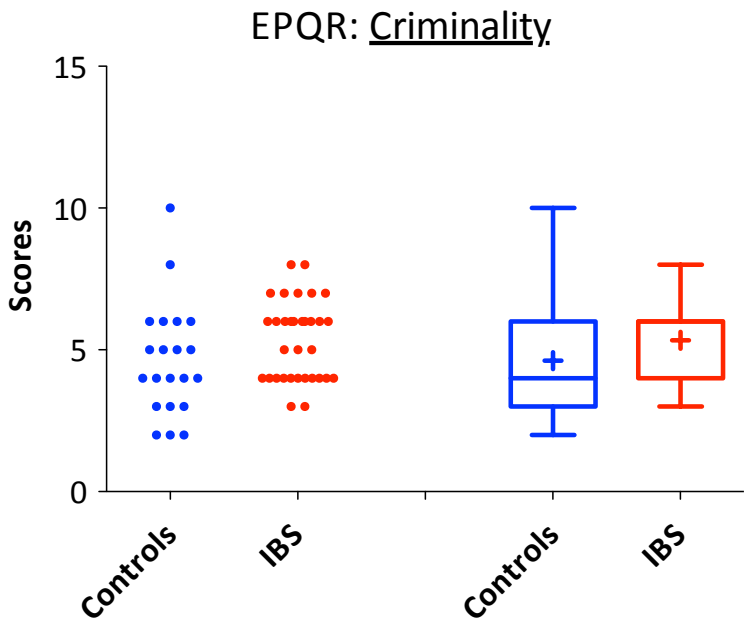


Table 3.1.7 Eysenck Personality Questionnaire- Revised: Criminality

Unpaired t test	
P value	0.1280
Mean ± SEM of Controls	4.619 ± 0.4336 N=21
Mean ± SEM of IBS	5.333 ± 0.2449 N=33
Difference between means	-0.7143 ± 0.4618
95% confidence interval	-1.642 to 0.2131

Analysis

The EPQ-R demonstrated statistical significance between groups for extraversion, neuroticism and lie. IBS patients were more introverted (i.e. less extrovert). Introversion is a state of chronic over-arousal in which one actively seeks a more relaxed, serene environment. IBS patients were more neurotic, which reflects a state of emotional instability, constant worrying and a tendency towards depression and anxiety. The lie scale is the concept of dissimulation, in which one pretends that things are better than they actually are.

The value of using the EPQ-R in this study is that few investigators have researched the personality traits of people with IBS. The EPQ-R has not been used before in an IBS study. Another point to make is that the increased neuroticism scores (with the inherent implication of increased tendencies to anxiety and depression) show good correlation with the Hospital Anxiety and Depression scores and Spielberger State Trait Anxiety Inventory scores.

Figure 3.1.8 Visceral Sensitivity Index

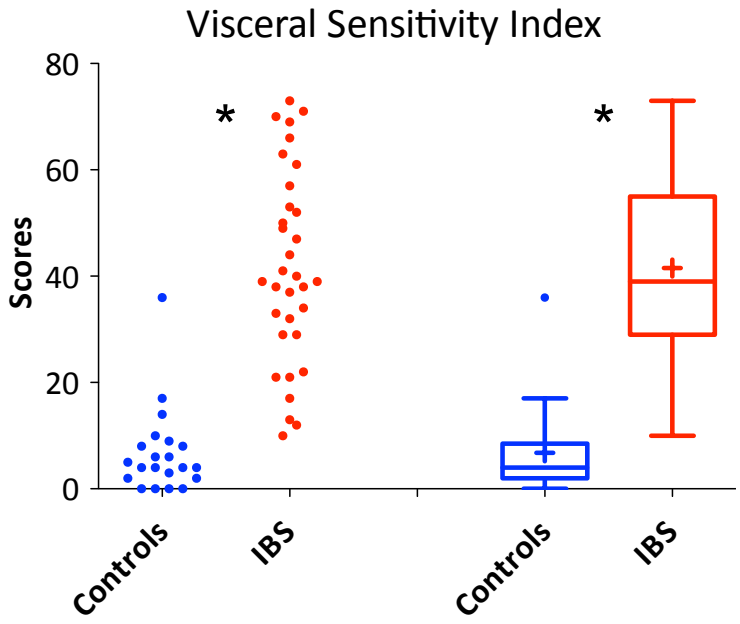


Table 3.1.8 Visceral Sensitivity Index

Unpaired t test	
P value	< 0.0001
Mean ± SEM of Controls	6.762 ± 1.765 N=21
Mean ± SEM of IBS	41.52 ± 3.169 N=33
Difference between means	-34.75 ± 4.226
95% confidence interval	-43.24 to -26.27

Scoring

15 questions with a maximum of 5 points per question, making a combined total of 75.

Analysis

The Visceral Sensitivity Index assesses anxiety related to specific gastrointestinal symptoms. Examples include being anxious about going into a new restaurant, being frightened about abdominal discomfort and being aware of abdominal symptoms during periods of stress etc. As a consequence, the IBS population was always going to score more highly the control population, as seen by the level of statistical significance that was attained. Of the 21 controls, 3 subjects scored over 10. In the IBS group, only 3 out of 33 scored less than 20, the average was 41.5 and the range spanned from 10 to 73. It is obvious that anxiety related to gastrointestinal symptoms, and the fear of losing control of one's bodily function (its ultimate expression being faecal incontinence) causes significant distress in IBS patients. Anecdotally, many of the IBS patients described how they always needed to know exactly where a toilet was when they were out, and some felt almost paralysed by the thought of having to leave their own homes.

Figure 3.1.9 Patient Health Questionnaire 15

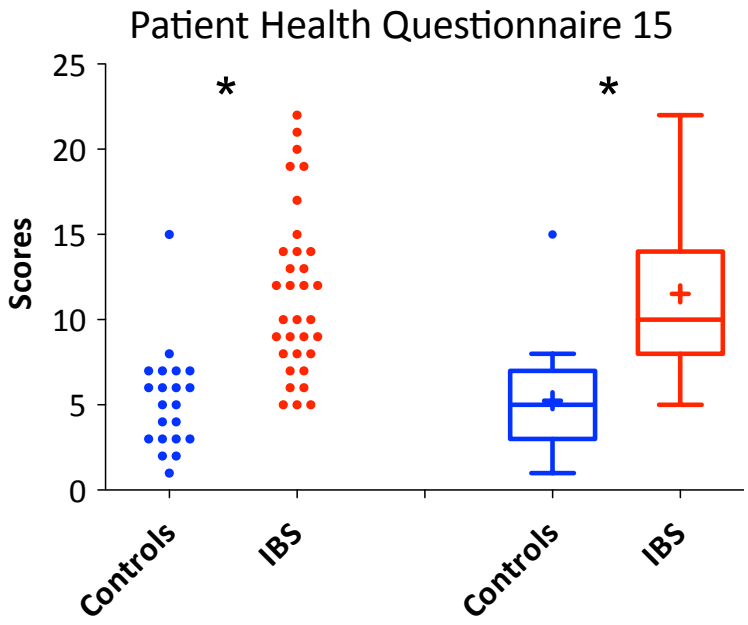


Table 3.1.9 Patient Health Questionnaire 15

Unpaired t test	
P value	< 0.0001
Mean ± SEM of Controls	5.238 ± 0.6543 N=21
Mean ± SEM of IBS	11.52 ± 0.8418 N=33
Difference between means	-6.277 ± 1.179
95% confidence interval	-8.646 to -3.909

Scoring

15 questions with a maximum score of 2 per question, giving a combined maximum of 30. A case is considered to have a score of 6 or more.

Analysis

The PHQ-15 data shows an increase in somatisation in IBS patients. The Control group mean was 5.24, within the normal range. This compared to a mean of 11.52 in the IBS group. High PHQ values cannot diagnose somatoform disorders, but measure somatic symptom severity. An interesting observation in studies which have used the PHQ-15 is that high scores are associated with excessive consumption of health care resources. Of course, IBS patients (like most other medical conditions) are divided into those cases that present to their doctors for investigation of their symptoms, and those that do not. However, in tertiary gastroenterology centres, it is not difficult to find a subgroup of IBS patients who are not satisfied until they have been subjected to every investigation to exclude organic pathology, and even then they find it difficult to accept the idea that their symptoms have a functional aetiology. Certainly, somatisation or the transference of internal psychological conflict into physical symptomatology, may have some influence in this behaviour.

Figure 3.1.10 Whiteley Index

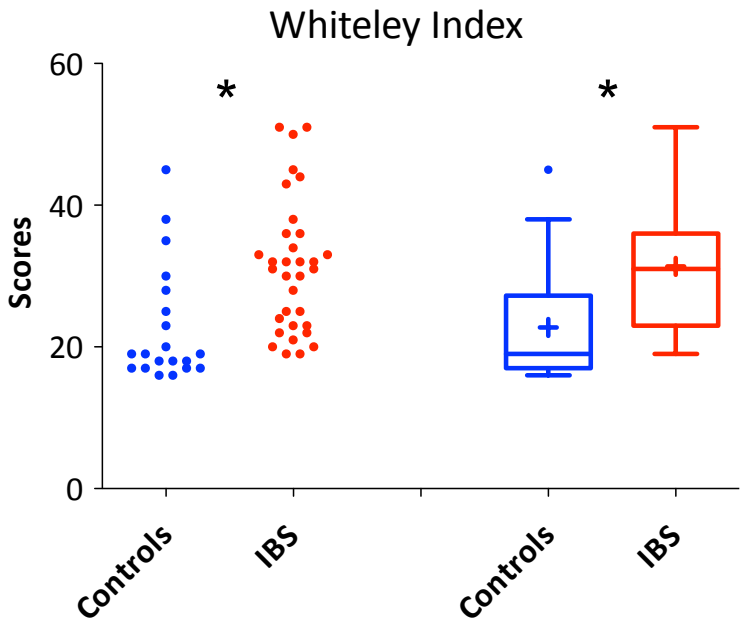


Table 3.1.10 Whiteley Index

Unpaired t test	
P value	0.0014
Mean ± SEM of Controls	22.75 ± 1.852 N=20
Mean ± SEM of IBS	31.36 ± 1.639 N=33
Difference between means	-8.614 ± 2.554
95% confidence interval	-13.74 to -3.482

Scoring

14 questions with between 1 and 5 scored per question to make a combined total of 70. Healthy people without health anxiety generally have a score of 21 +/- 7 (14 to 28). Patients with hypochondria are found to have a score of 44 +/- 11 (32 to 55).

Analysis

The Whiteley Index demonstrates good statistical significance between the groups. With reference to the control population 3 subjects had scores at or above the threshold of 32 to imply some degree of hypochondriasis. The control range was 16 to 45. Sixteen of 33 IBS scored 32 with a group range of 19 to 51. The IBS group mean was 31.36 just short of this threshold. In addition to disease conviction and disease phobia, the Whiteley Index explores somatic preoccupation, thereby tying in with the data observed from the PHQ-15.

DAY 1 TO 3 PSYCHOLOGICAL TESTS

The following psychological tests were completed on each of the 3 days of CEP testing. They were chosen because they were powered to observe volatile psychological factors i.e. those factors that change over short periods of time e.g. situational anxiety.

Figure 3.1.11 Hospital Anxiety and Depression: Anxiety Score

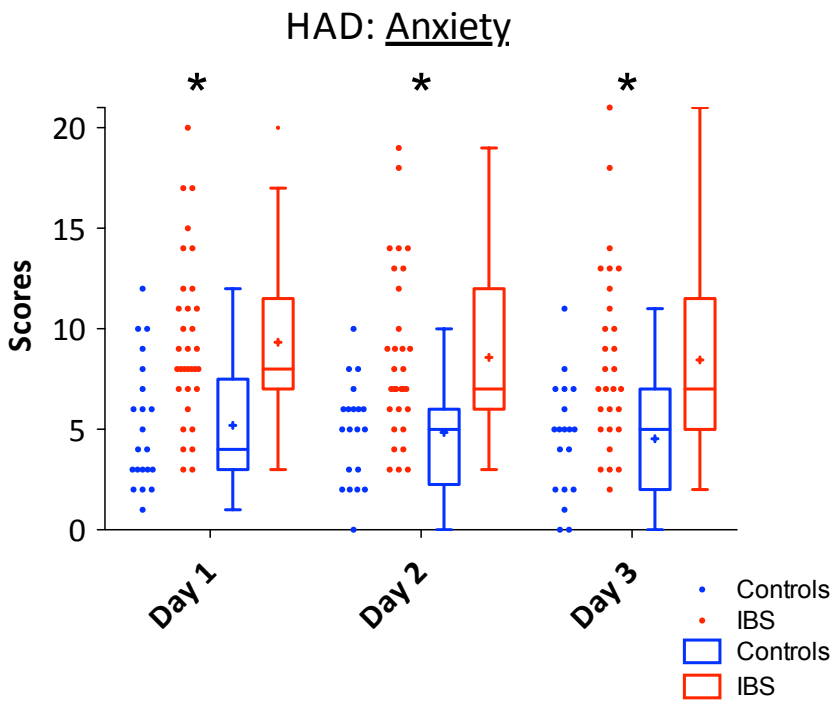


Table 3.1.11 Hospital Anxiety and Depression: Anxiety Score

Unpaired t test	Day 1	Day 2	Day 3
P value	0.0002	0.0008	0.0017
Mean ± SEM of Controls	5.190 ± 0.6818 N=21	4.850 ± 0.5632 N=20	4.526 ± 0.6595 N=19
Mean ± SEM of IBS	9.333 ± 0.7186 N=33	8.581 ± 0.7589 N=31	8.448 ± 0.8446 N=29
Difference between means	-4.143 ± 1.053	-3.731 ± 1.049	-3.922 ± 1.174
95% confidence interval	-6.259 to -2.027	-5.842 to -1.620	-6.287 to -1.557

Scoring

7 questions with scores between 0 and 3 producing a combined total of 21. Non-cases score between 0 & 7; possible cases score between 8 & 10; probable cases score between 11 & 21.

Analysis

The HAD-Anxiety scores recorded in the IBS group were repeatedly and significantly more than the Control group. The IBS mean 3 day averages spanned between 8.4 and 9.3, identifying *possible* anxiety cases. The Control group mean was 5.19 on Day 1, dropping to 4.52 by Day 3. Interestingly, 2 Controls on Day 1 were identified as possible cases, and 2 more were identified as probable cases. This distinction persisted in 2 of the Controls over the next 2 days (with the other 2 dropping into the normal ranges).

Figure 3.1.12 Hospital Anxiety and Depression: Depression Score

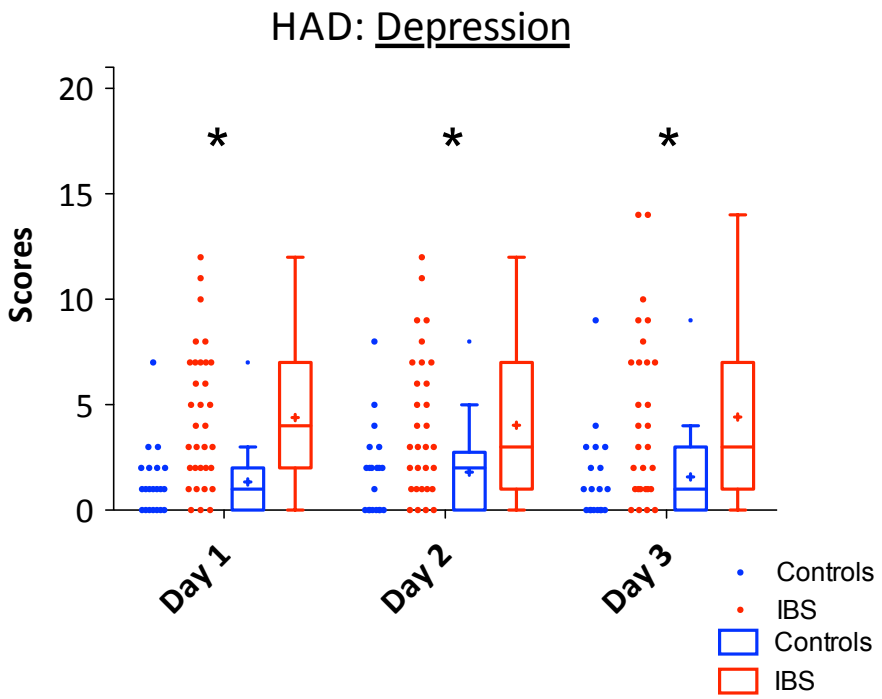


Table 3.1.12 Hospital Anxiety and Depression: Depression Score

Unpaired t test	Day 1	Day 2	Day 3
P value	0.0002	0.0095	0.0082
Mean ± SEM of Controls	1.333 ± 0.3541 N=21	1.800 ± 0.4451 N=21	1.579 ± 0.5091 N=19
Mean ± SEM of IBS	4.394 ± 0.5673 N=33	4.032 ± 0.6090 N=31	4.414 ± 0.7584 N=29
Difference between means	-3.061 ± 0.7670	-2.232 ± 0.8271	-2.835 ± 1.026
95% confidence interval	-4.601 to -1.520	-3.895 to -0.5695	-4.902 to -0.7681

Scoring

7 questions with scores between 0 and 3 producing a combined total of 21. Non-cases score between 0 & 7; possible cases score between 8 & 10; probable cases score between 11 & 21.

Analysis

The HAD-Depression looks for depressive tendencies, and shares the same scoring system as the HAD-Anxiety. Both group means fell within the range of normality (i.e. less than 8), but even so, the IBS patients were found to have more depressive tendencies than their healthy counterparts. No possible or probable cases were identified in the Control group over the 3 days. The range of scores in the IBS group was 0 and 12 (Days 1 and 2), and 0 and 14 (Day 3) proving that certain individuals were affected to a telling degree.

Figure 3.1.13 Spielberger State Trait Anxiety Inventory: State Anxiety

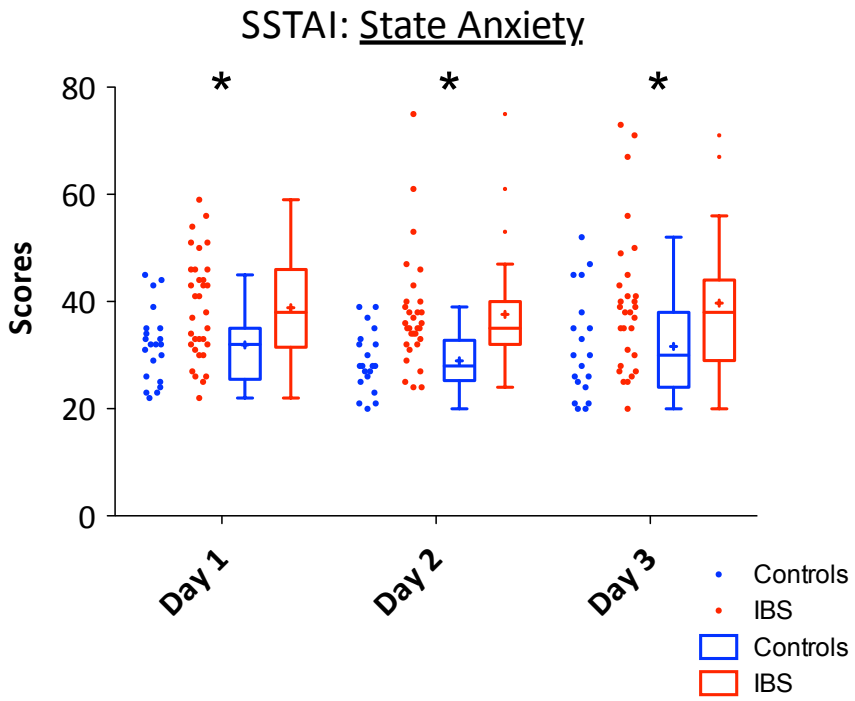


Table 3.1.13 Spielberger State Trait Anxiety Inventory: State Anxiety

Unpaired t test	Day 1	Day 2	Day 3
P value	0.0062	0.0014	0.0297
Mean ± SEM of Controls	31.90 ± 1.489 N=21	28.95 ± 1.274 N=20	31.63 ± 2.265 N=19
Mean ± SEM of IBS	38.85 ± 1.691 N=33	37.61 ± 1.884 N=31	39.69 ± 2.494 N=29
Difference between means	-6.944 ± 2.434	-8.663 ± 2.564	-8.058 ± 3.590
95% confidence interval	-11.83 to -2.056	-13.82 to -3.505	-15.29 to -0.8247

Scoring

20 questions scored between 1 and 4, making a combined total of 80. Cases score more than 35.

Analysis

The Spielberger State Anxiety Index is powered to detect situational anxiety. It offers to identify those subjects who demonstrate ongoing anxiety. Its inclusion was brought about by the desire to see if there was any difference between the study groups when both were faced with the same unknown entity, namely rectal electrical stimulation and CEP harvesting. Surprisingly statistical significance was achieved even at Day 1with a mean IBS score of 38.8 (compared to 31.9 in the Controls). At no point did the State Anxiety drop below the case threshold of 35 in the IBS group. This suggests that IBS patients mount a greater anxiety response to the same perceived threat (the electrical stimulation), and that this does not diminish over time even with increasing familiarity of the test protocol.

Figure 3.1.14 Spielberger State Trait Anxiety Inventory: Trait Anxiety

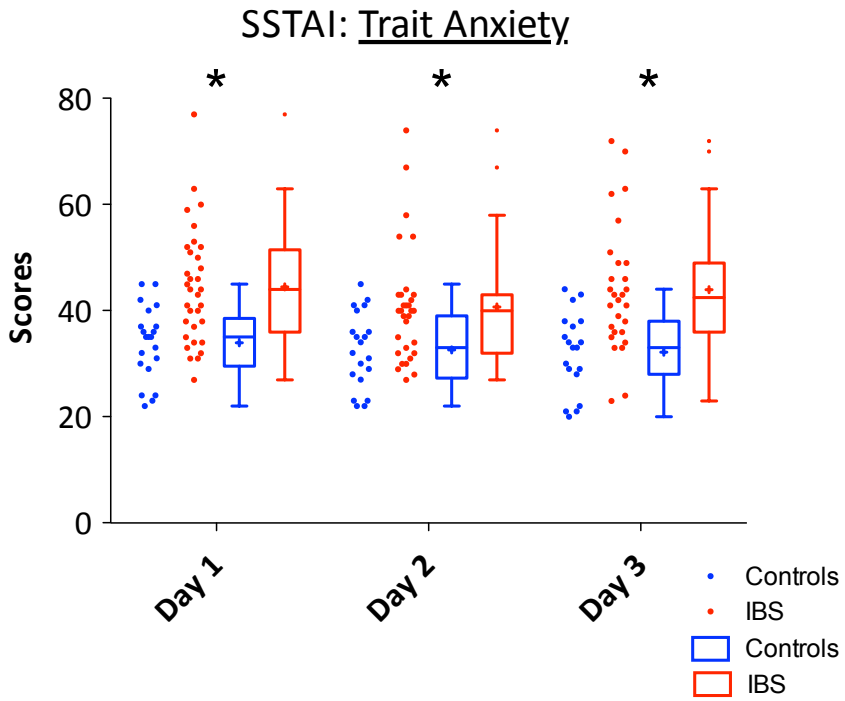


Table 3.1.14 Spielberger State Trait Anxiety Inventory: Trait Anxiety

Unpaired t test	Day 1	Day 2	Day 3
P value	0.0002	0.0051	0.0004
Mean ± SEM of Controls	33.90 ± 1.496 N=21	32.60 ± 1.588 N=20	32.16 ± 1.714 N=19
Mean ± SEM of IBS	44.48 ± 1.882 N=33	40.71 ± 1.966 N=31	43.96 ± 2.285 N=28
Difference between means	-10.58 ± 2.648	-8.110 ± 2.765	-11.81 ± 3.118
95% confidence interval	-15.90 to -5.261	-13.67 to -2.548	-18.09 to -5.522

Scoring

20 questions scored between 1 and 4, making a combined total of 80. Cases score more than 35.

Analysis

The Spielberger Trait Anxiety Index examines for one's general propensity to anxiety, essentially defining an anxiety characteristic. The Control group scored persistently below the case threshold of 35, whereas the IBS group did not fall below 40 over the 3 days. The Trait Anxiety score is more time independent than the State Anxiety. However both indexes showed little variation in either group over the 3 separate days.

Figure 3.1.15 Visual Descriptor Visual Analogue Scale: Unpleasantness of Symptoms

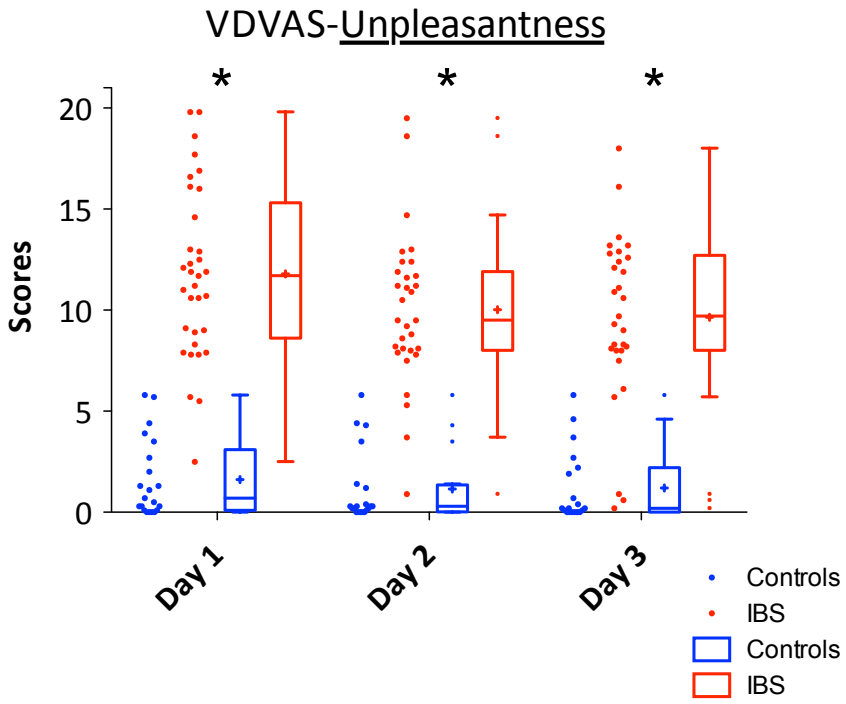


Table 3.1.15 Visual Descriptor Visual Analogue Scale: Unpleasantness of Symptoms

Unpaired t test	Day 1	Day 2	Day 3
P value	< 0.0001	< 0.0001	< 0.0001
Mean ± SEM of Controls	1.619 ± 0.4220 N=21	1.145 ± 0.4025 N=20	1.200 ± 0.4106 N=19
Mean ± SEM of IBS	11.78 ± 0.7361 N=33	10.02 ± 0.6769 N=31	9.631 ± 0.7841 N=29
Difference between means	-10.17 ± 0.9847	-8.871 ± 0.9049	-8.431 ± 1.027
95% confidence interval	-12.14 to -8.188	-10.69 to -7.051	-10.50 to -6.362

Scoring

The VDVAS is a 20cm vertical column with various descriptors outlined at defined distances from the baseline at 0cm. Increasing distance from baseline indicates increased severity of the unpleasantness of gastrointestinal symptoms.

Analysis

The VDVAS for the unpleasantness of gastrointestinal symptoms produces a clear demarcation between IBS and Control groups. This is hardly surprising given the fact the Control group by definition is asymptomatic. However it afforded a snapshot of the perception of gastrointestinal symptomatology, and the generation of continuous data.

Figure 3.1.16 Visual Descriptor Visual Analogue Scale: Intensity of Symptoms

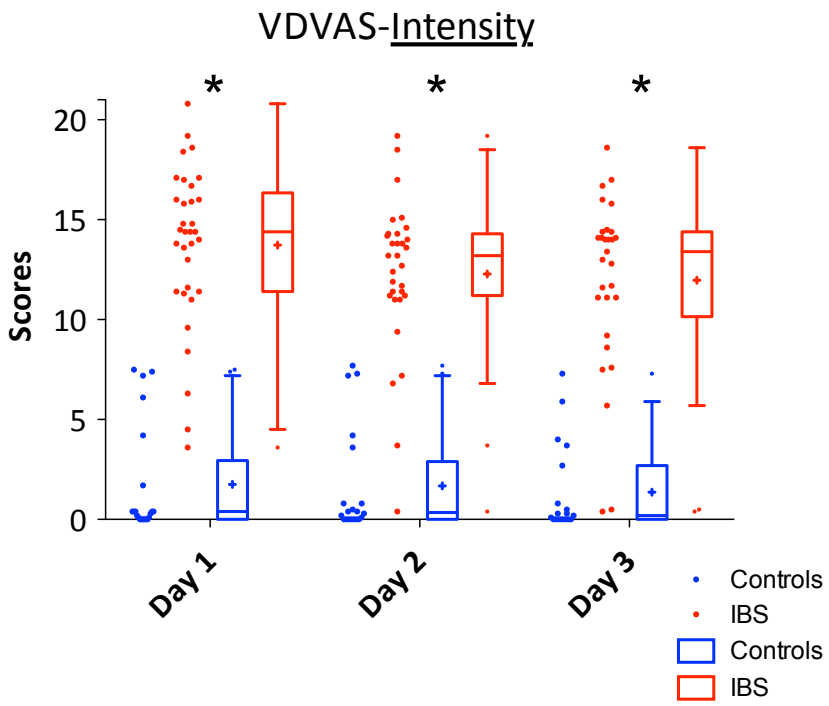


Table 3.1.16 Visual Descriptor Visual Analogue Scale: Intensity of Symptoms

Unpaired t test	Day 1	Day 2	Day 3
P value	< 0.0001	< 0.0001	< 0.0001
Mean ± SEM of Controls	1.752 ± 0.6115 N=21	1.675 ± 0.6089 N=20	1.358 ± 0.5163 N=19
Mean ± SEM of IBS	13.73 ± 0.6943 N=33	12.29 ± 0.6890 N=31	11.97 ± 0.8123 N=29
Difference between means	-11.98 ± 0.9991	-10.62 ± 0.9888	-10.61 ± 1.090
95% confidence interval	-13.99 to -9.974	-12.60 to -8.626	-12.80 to -8.412

Scoring

The VDVAS is a 20cm vertical column with various descriptors outlined at defined distances from the baseline at 0cm. Increasing distance from baseline indicates increased severity of the intensity of gastrointestinal symptoms.

Analysis

The VDVAS for gastrointestinal intensity is constructed in an identical fashion to the VDVAS for unpleasantness, with the exception that different visual descriptors are used. Like its sibling, the VDVAS for intensity of gastrointestinal symptoms produces a clear separation between the two groups.

ANORECTAL SENSITIVITY AND CORTICAL EVOKED POTENTIALS

The following section comprises the anorectal physiology data, featuring the sensory and pain thresholds; analysis of the morphology of the CEPs; the assessment of the CEPs peaks with regards to latency and amplitude; and Tables 3.1.22b to d show the comparison of the peak latencies and amplitudes for the Control group over the 3 days of testing.

Figure 3.1.17 Sensory Thresholds

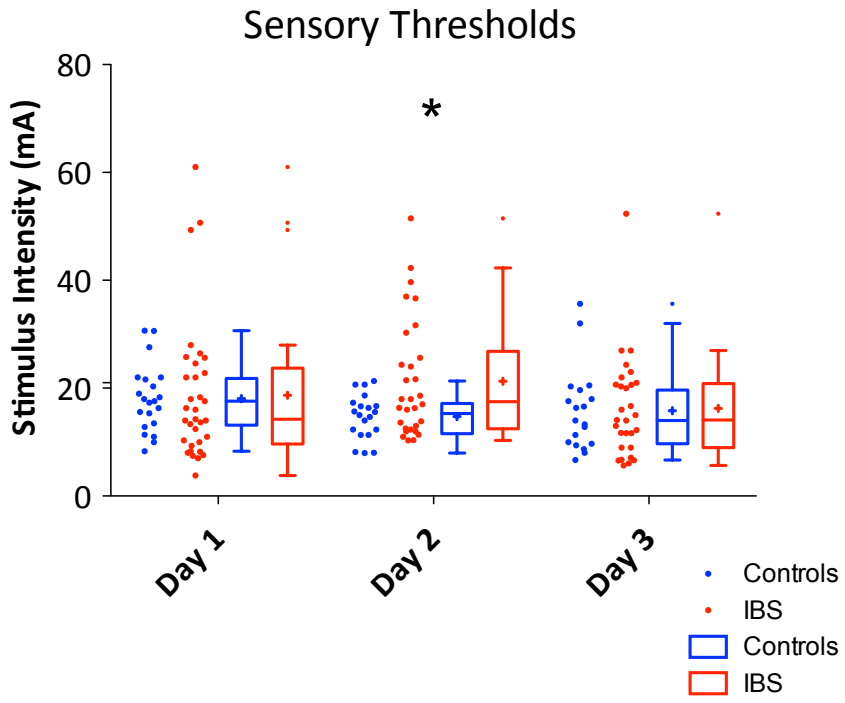


Table 3.1.17 Sensory Thresholds

Unpaired t test	Day 1	Day 2	Day 3
P value	0.8347	0.0139	0.8717
Mean ± SEM of Controls	18.06 ± 1.360 N=21	14.73 ± 0.9137 N=20	15.82 ± 1.767 N=19
Mean ± SEM of IBS	18.70 ± 2.277 N=33	21.29 ± 2.000 N=30	16.24 ± 1.772 N=29
Difference between means	-0.6418 ± 3.061	-6.560 ± 2.568	-0.4252 ± 2.618
95% confidence interval	-6.789 to 5.505	-11.73 to -1.392	-5.699 to 4.849

Analysis

Mean values for sensory thresholds were similar in both groups over Days 1 and 3. Day 2 produced a separation between the groups with an IBS mean of 21.29mA and a Control mean of 14.73mA. With reference to the scattergrams, it is quite clear that there were a few IBS outliers who were skewing the distribution to the right. Even so a higher sensory threshold reflects either a hyposensitive state (not consistent with the concept of visceral hypersensitivity) or poor contact of the electrode to the rectal wall.

Figure 3.1.18 Pain Thresholds

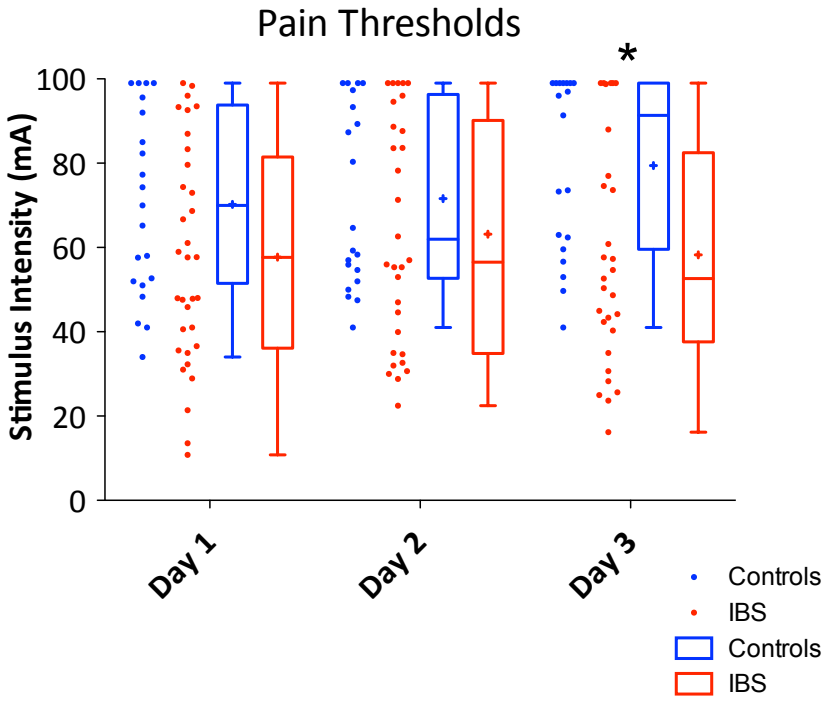


Table 3.1.18 Pain Thresholds

Unpaired t test	Day 1	Day 2	Day 3
P value	0.0735	0.2463	0.0058
Mean ± SEM of Controls	70.21 ± 4.793 N=21	71.61 ± 4.814 N=20	79.45 ± 4.839 N=19
Mean ± SEM of IBS	57.72 ± 4.513 N=33	63.19 ± 4.893 N=30	58.23 ± 5.011 N=29
Difference between means	12.48 ± 6.833	8.420 ± 7.173	21.22 ± 7.334
95% confidence interval	-1.241 to 26.20	-6.017 to 22.86	6.440 to 35.99

Analysis

The pain threshold denotes the stimulus intensity that confers a painful response. Comparison of the 2 groups shows a lower pain threshold in IBS patients over the three days, reaching statistical significance on Day 3. This is due to an increase in the pain thresholds in the Control group rather than a corresponding drop in the pain thresholds in the IBS group. The IBS thresholds stay largely unchanged over the three days. There is a much larger range of thresholds in the IBS patients, with values recorded between 10.83mA and 99mA on the first day, contrasting with 34mA and 99mA in the control group. The rise in pain thresholds by the Control group on Day 3 may be explained by the fact that the Control group has started to habituate to the testing protocol allowing a greater current to be applied. Symptom-generation and propagation in IBS may be partially driven by the inability to relax in the face of familiar or chronic stressors.

Figure 3.1.19 A Cortical evoked potential recorded after a single stimulation from a Control subject

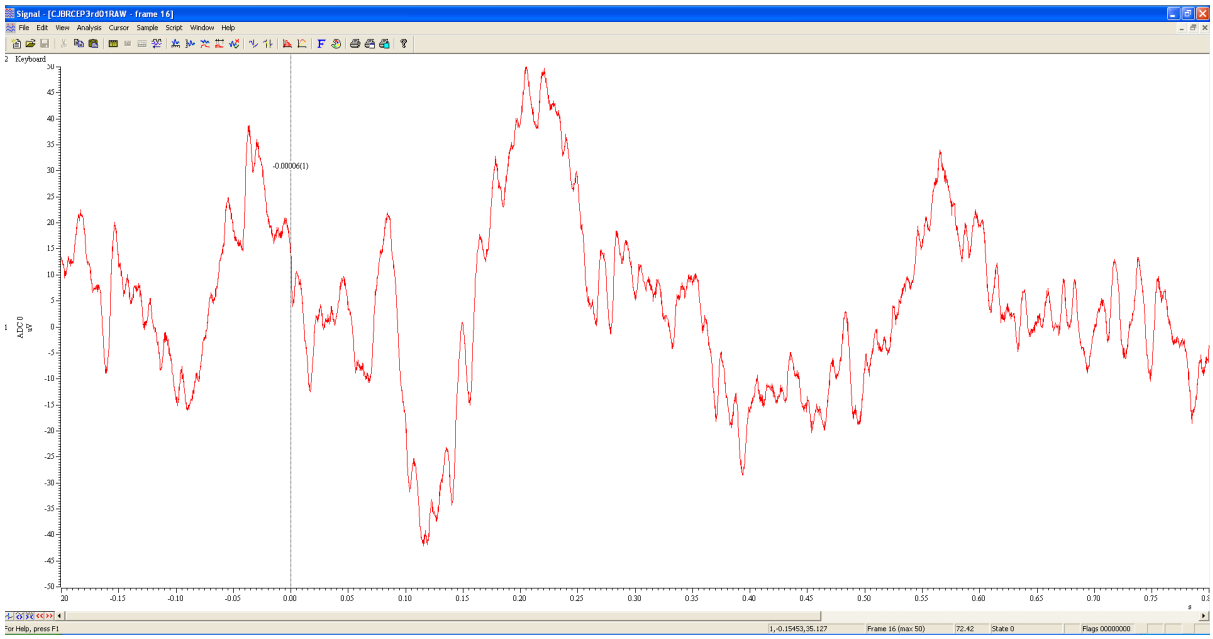


Figure 3.1.20 A Cortical evoked potential recorded after 1 cycle of 50 stimulations from a Control subject

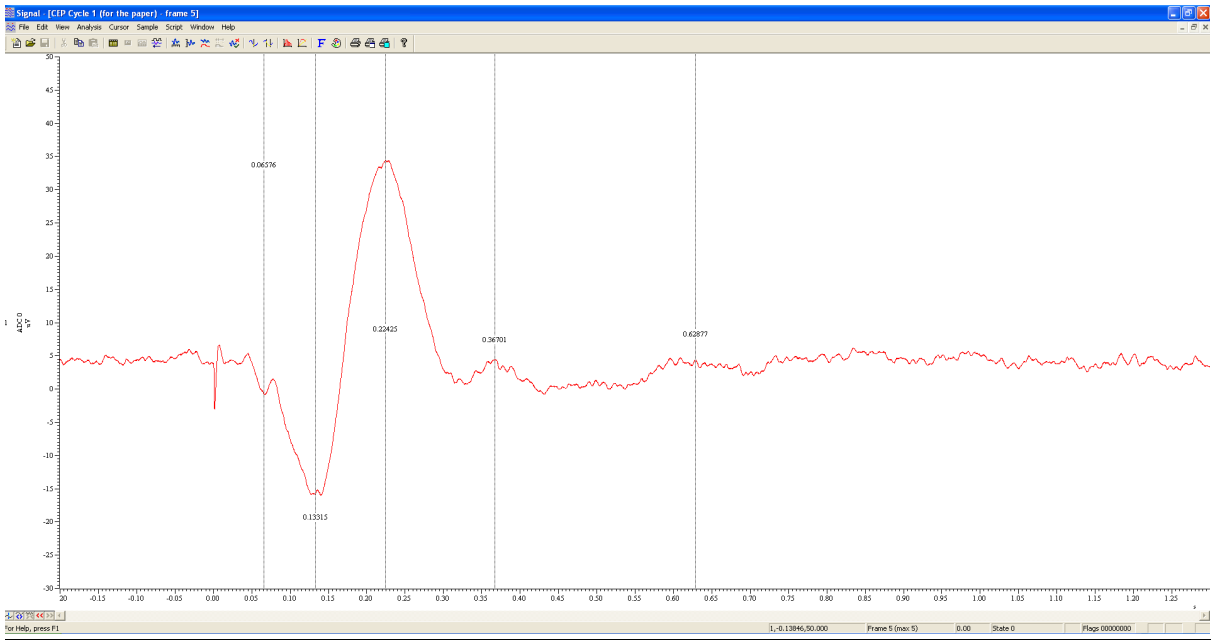


Figure 3.1.21 Cortical evoked potentials recorded over 4 cycles of 50 electrical stimulations from a Control subject

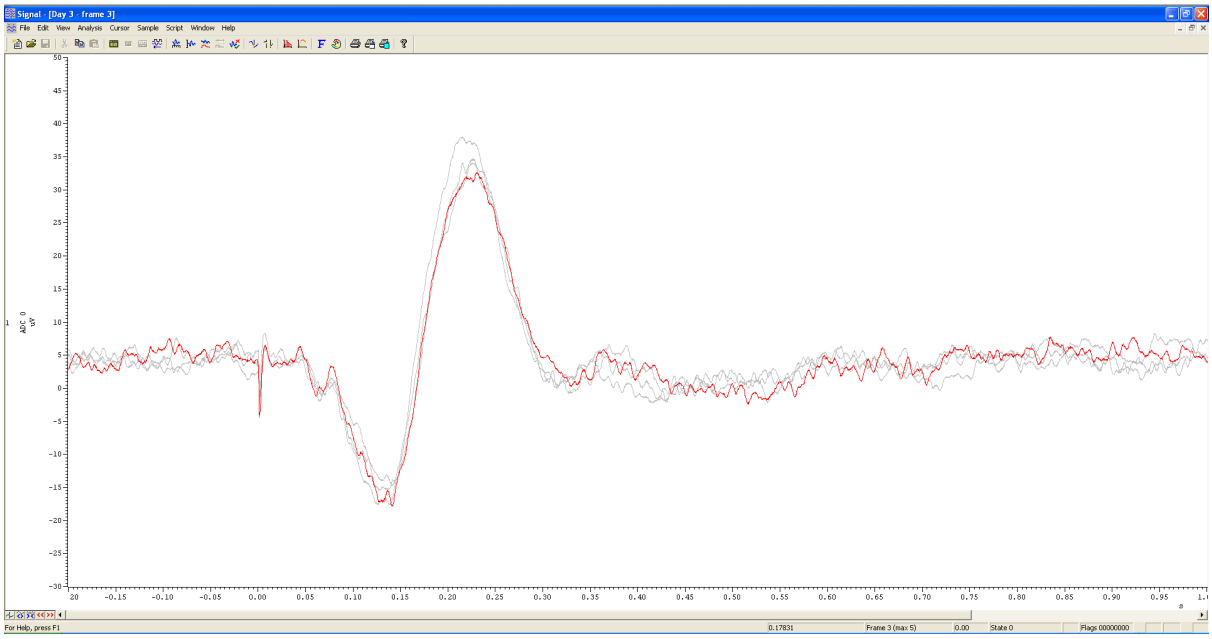
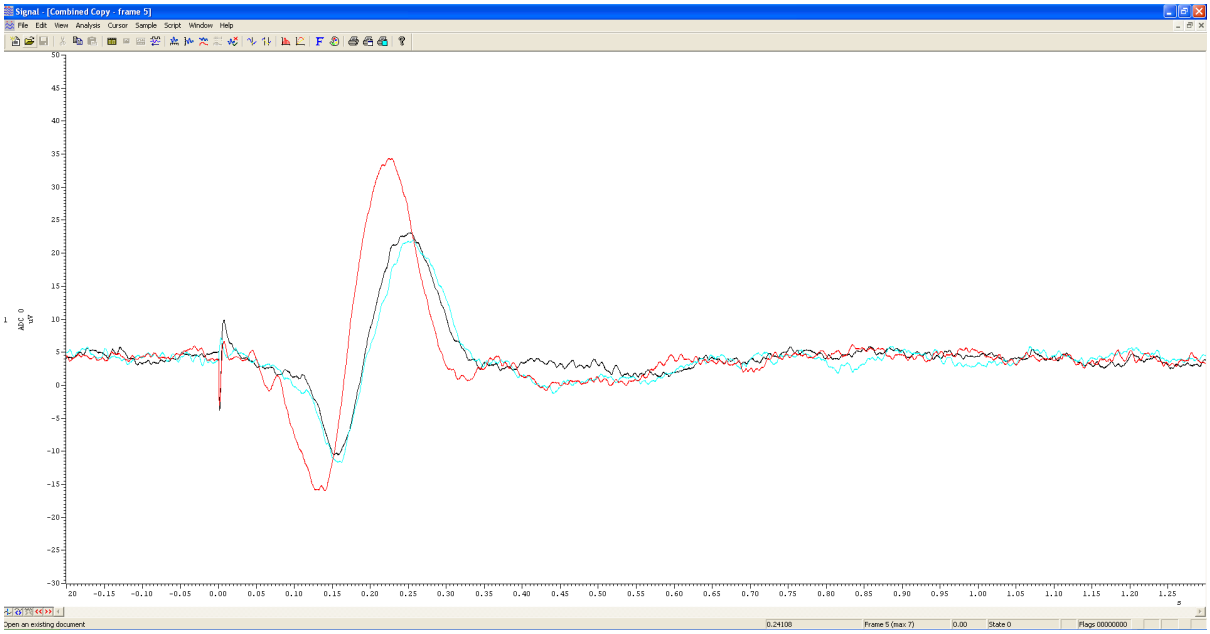


Figure 3.1.22 Averaged cortical evoked potentials recorded over 3 separate days from a Control subject



Analysis

Figure 3.1.19 shows how difficult it is to analyse a CEP in isolation. Each trace is readily susceptible to movement artefact, and it is only after the averaging of multiple frames that the true morphology of the cortical response is seen (Figure 3.1.20). The vertical dotted lines in this figure reveal the distribution of the separate peaks so that the latencies and amplitudes can be calculated.

The intra-day reproduction of the cortical response over a series of 4 separate cycles of 50 stimulations is clearly evident in Figure 3.1.21. The inter-day comparisons are seen in Figure 3.1.22. The black and green graphs represent testing on Day 1 and 2. The Day 3 graph is coloured in red. For this particular control subject, the Spielberger State Anxiety steadily fell from the initial peak of 33 on Day 1 to 27 on Day 2 and 26 on Day 3. The stimulus intensity increased from 33.25mA on Day 1 to 58.6mA on Day 3. This suggests that increased familiarity with the testing produced a more relaxed subject, and the opportunity to increase the stimulus intensity resulting in a more prominent cortical response.

INTRA-DAY AND INTER-DAY VARIABILITY OF CORTICAL EVOKED POTENTIALS IN THE CONTROL GROUP

Table 3.1.22b One Way ANOVA of the mean difference of anorectal sensitivities, CEP peak latencies and CEP peak amplitudes between Day 2 and Day 1 in the Control group

	Mean difference	**95% CI**	**P-value**
Sensory Threshold (mA)	-3.33	(-7.18, 0.52)	0.089
Pain Threshold	1.41	(-12.07, 14.89)	0.8351
	Mean difference	**95% CI**	**P-value**
Peak 1 Latency (ms)	0.64	(-9.72, 11.00)	0.9018
Peak 2 Latency	-5.14	(-18.05, 7.78)	0.4288
Peak 3 Latency	-5.27	(-19.83, 9.28)	0.471
Peak 4 Latency	-11.71	(-39.42, 16.01)	0.4011
Peak 5 Latency	-9.58	(-75.45, 56.28)	0.7717
	Mean difference	**95% CI**	**P-value**
Peak 1 Amplitude (mV)	0.63	(-2.45, 3.72)	0.6821
Peak 2 Amplitude	2.65	(-4.82, 10.12)	0.4803
Peak 3 Amplitude	3.52	(-2.47, 9.51)	0.2439
Peak 4 Amplitude	0.49	(-2.40, 3.38)	0.7354

Table 3.1.22c One Way ANOVA of the mean difference of anorectal sensitivities, CEP peak latencies and CEP peak amplitudes between Day 3 and Day 1 in the Control group

	Mean difference	**95% CI**	**P-value**
Sensory Threshold (mA)	-2.24	(-6.15, 1.66)	0.2545
Pain Threshold	9.24	(-4.42, 22.91)	0.1808
	Mean difference	**95% CI**	**P-value**
Peak 1 Latency (ms)	-0.75	(-11.11, 9.61)	0.8855
Peak 2 Latency	-6.56	(-19.47, 6.35)	0.3133
Peak 3 Latency	-8.71	(-23.27, 5.85)	0.2357
Peak 4 Latency	-16.84	(-44.56, 10.87)	0.2286
Peak 5 Latency	0.45	(-65.42, 66.32)	0.9892
	Mean difference	**95% CI**	**P-value**
Peak 1 Amplitude (mV)	-0.05	(-3.14, 3.04)	0.9744
Peak 2 Amplitude	3.77	(-3.69, 11.24)	0.3155
Peak 3 Amplitude	5.56	(-0.43, 11.54)	0.0683
Peak 4 Amplitude	1.46	(-1.43, 4.35)	0.3146

Table 3.1.22d One Way ANOVA of the mean difference of anorectal sensitivities, CEP peak latencies and CEP peak amplitudes between Day 3 and Day 2 in the Control group

	Mean difference	**95% CI**	**P-value**
Sensory Threshold (mA)	1.08	(-2.89, 5.06)	0.5834
Pain Threshold	7.84	(-6.00, 21.67)	0.2586
	Mean difference	**95% CI**	**P-value**
Peak 1 Latency (ms)	-1.39	(-12.01, 9.23)	0.7922
Peak 2 Latency	-1.42	(-14.97, 12.13)	0.8328
Peak 3 Latency	-3.44	(-16.81, 9.93)	0.6054
Peak 4 Latency	-5.14	(-32.68, 22.40)	0.7074
Peak 5 Latency	10.03	(-61.05, 81.11)	0.7764
	Mean difference	**95% CI**	**P-value**
Peak 1 Amplitude (mV)	-0.68	(-4.01, 2.65)	0.6793
Peak 2 Amplitude	1.13	(-6.98, 9.23)	0.7797
Peak 3 Amplitude	2.04	(-4.33, 8.40)	0.5205
Peak 4 Amplitude	0.97	(-2.24, 4.19)	0.5428

Analysis

Tables 3.1.22b, 3.1.22c and 3.1.22d compare control data pertaining to sensory and pain thresholds, and CEP peak amplitudes and latencies. One Way ANOVA could supported the null hypothesis concluding that the CEP protocol produced reproducible and reliable data over different days of testing in the control group i.e. the CEP data was no different on different days of testing . Without this important finding, it would have been impossible to deploy the CEP test paradigm on the IBS group.

Analysis of the Cortical Evoked Potentials between Controls and IBS

The following series of graphs shows the comparison of the individual CEP components between the Control and IBS groups.

Figure 3.1.23 Peak 1 Latency over 3 days

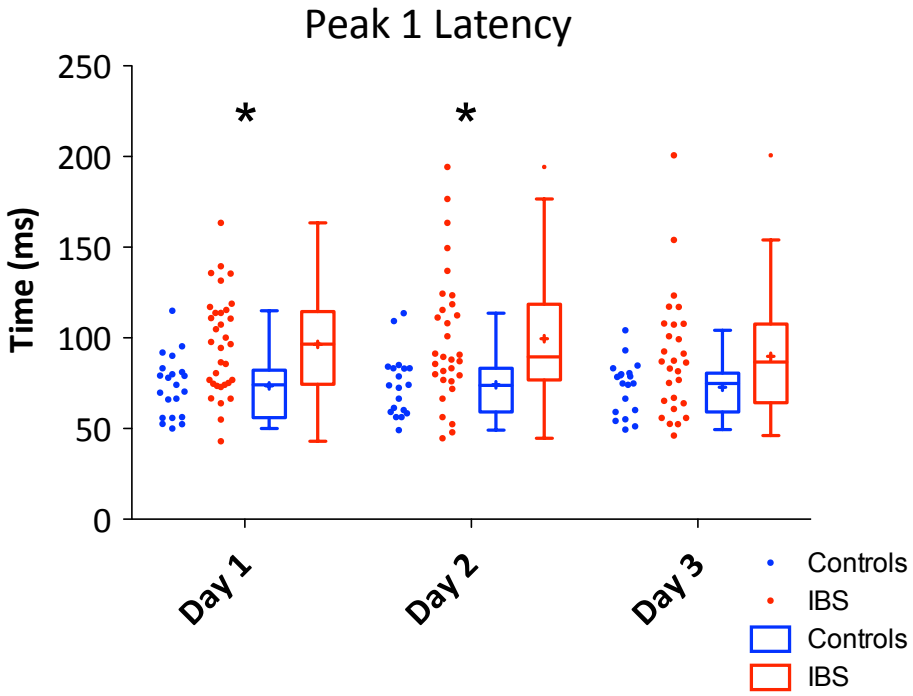


Table 3.1.23 Peak 1 Latency over 3 days

Unpaired t test	Day 1	Day 2	Day 3
P value	0.0012	0.0067	0.1443
Mean ± SEM of Controls	73.49 ± 3.641 N=21	74.13 ± 3.987 N=19	72.74 ± 3.393 N=19
Mean ± SEM of IBS	96.35 ± 4.797 N=33	99.52 ± 6.550 N=31	107.8 ± 18.90 N=29
Difference between means	-22.86 ± 6.691	-25.38 ± 8.954	-35.05 ± 23.60
95% confidence interval	-36.30 to -9.424	-43.41 to -7.364	-82.59 to 12.49

Figure 3.1.24 Peak 2 Latency over 3 days

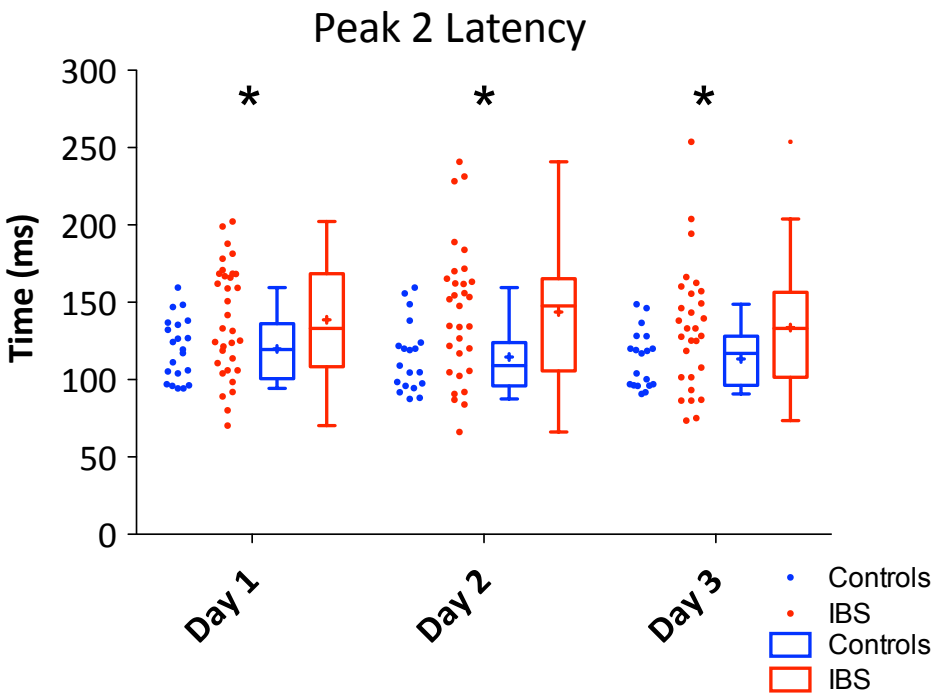


Table 3.1.24 Peak 2 Latency over 3 days

Unpaired t test	Day 1	Day 2	Day 3
P value	0.0311	0.0100	0.0461
Mean ± SEM of Controls	119.8 ± 4.347 N=21	114.7 ± 5.170 N=19	113.3 ± 4.234 N=19
Mean ± SEM of IBS	138.8 ± 6.211 N=33	143.6 ± 7.801 N=31	133.6 ± 7.503 N=29
Difference between means	-18.93 ± 8.540	-28.92 ± 10.78	-20.31 ± 9.908
95% confidence interval	-36.08 to -1.773	-50.62 to -7.226	-40.28 to -0.3519

Figure 3.1.25 Peak 3 Latency over 3 days

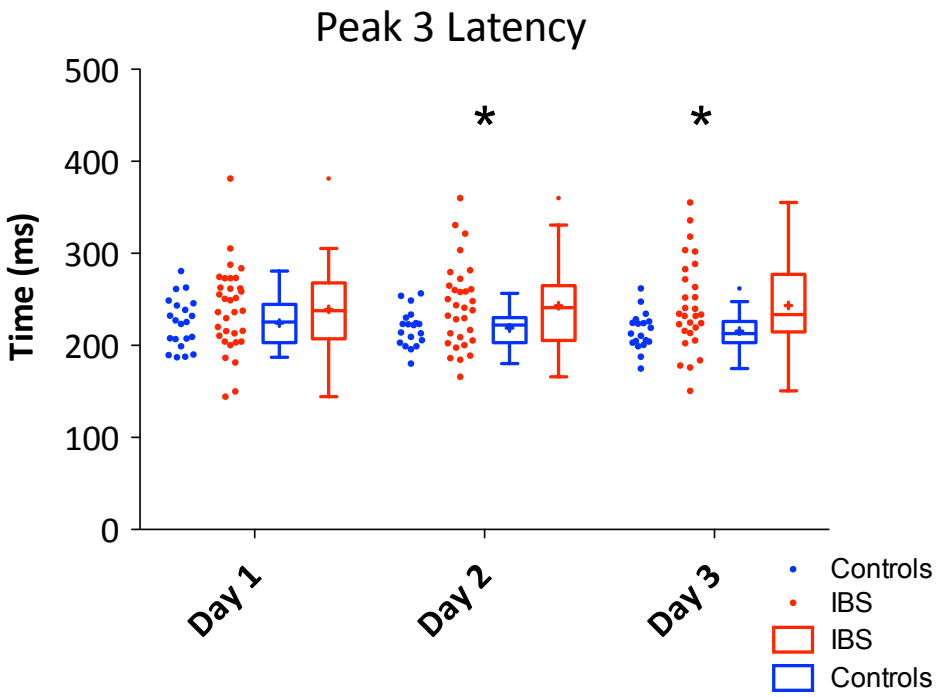


Table 3.1.25 Peak 3 Latency over 3 days

Unpaired t test	Day 1	Day 2	Day 3
P value	0.1828	0.0346	0.0229
Mean ± SEM of Controls	224.2 ± 5.903 N=21	218.9 ± 4.641 N=19	215.5 ± 4.682 N=19
Mean ± SEM of IBS	239.2 ± 8.004 N=33	243.1 ± 8.183 N=31	243.3 ± 9.051 N=29
Difference between means	-14.99 ± 11.10	-24.14 ± 11.10	-27.87 ± 11.84
95% confidence interval	-37.29 to 7.310	-46.48 to -1.806	-51.72 to -4.014

Figure 3.1.26 Peak 4 Latency over 3 days

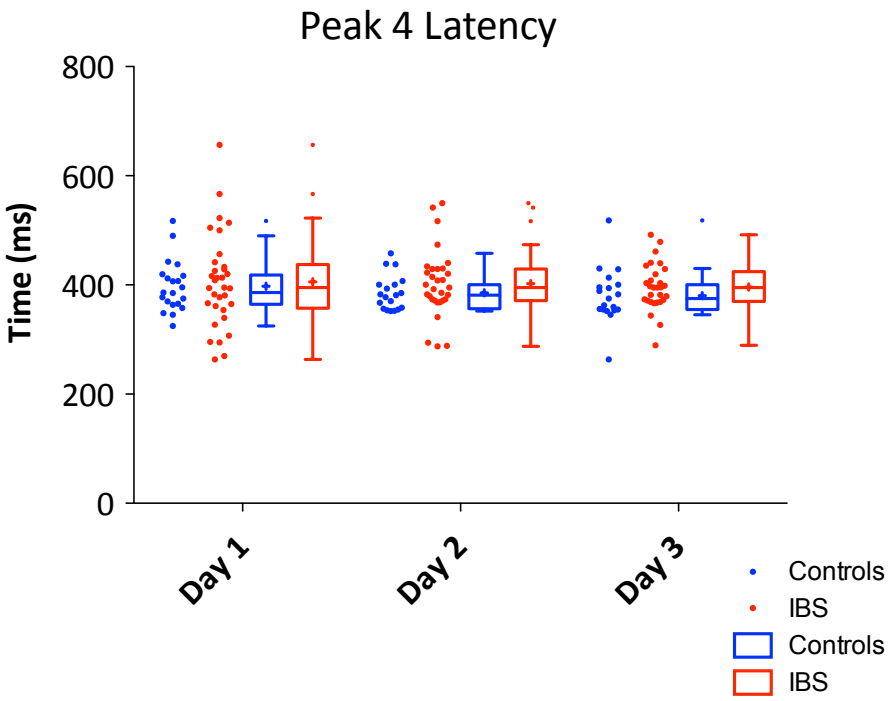


Table 3.1.26 Peak 4 Latency over 3 days

Unpaired t test	Day 1	Day 2	Day 3
P value	0.6833	0.2806	0.2584
Mean ± SEM of Controls	397.4 ± 10.22 N=21	385.7 ± 7.297 N=19	380.6 ± 11.45 N=19
Mean ± SEM of IBS	405.8 ± 14.84 N=33	402.5 ± 11.09 N=31	396.1 ± 8.015 N=29
Difference between means	-8.348 ± 20.35	-16.71 ± 15.32	-15.51 ± 13.55
95% confidence interval	-49.22 to 32.52	-47.54 to 14.11	-42.82 to 11.80

Figure 3.1.27 Peak 5 Latency over 3 days

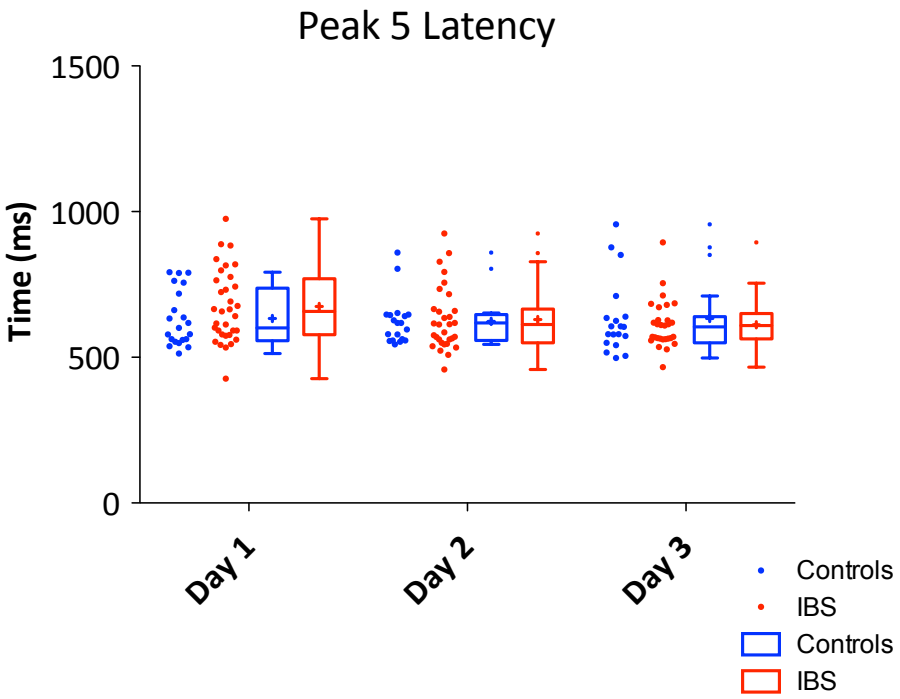


Table 3.1.27a Peak 5 Latency over 3 days

Unpaired t test	**Day 1**	**Day 2**	**Day 3**
P value	0.2023	0.8319	0.4858
Mean ± SEM of Controls	633.0 ± 20.92 N=21	623.4 ± 19.03 N=19	633.4 ± 29.43 N=19
Mean ± SEM of IBS	674.3 ± 21.72 N=33	629.7 ± 19.75 N=31	612.1 ± 15.30 N=29
Difference between means	-41.28 ± 31.96	-6.264 ± 29.34	21.32 ± 30.35
95% confidence interval	-105.5 to 22.92	-65.32 to 52.79	-39.81 to 82.46

Table 3.1.27b Summary of CEP Peak Latency Data

One Way ANOVA Comparison of Means: IBS vs. Controls

	Day 1				Day 2				Day 3			
(ms)	**Controls**	**IBS**	**Mean Δ**	**P-value**	**Controls**	**IBS**	**Mean Δ**	**P-value**	**Controls**	**IBS**	**Mean Δ**	**P-value**
Peak 1 L	73.5	96.35	22.8	0.0012	74.1	99.5	25.38	0.006	72.7	107.8	35.5	0.14
Peak 2 L	119.85	138.7	18.9	0.031	114.7	143.6	28.9	0.01	113.3	133.6	20.3	0.04
Peak 3 L	224.2	239.2	14.99	0.18	218.9	243.1	24.1	0.03	215.5	243.3	27.9	0.022
Peak 4 A	397.4	405.8	8.35	0.68	385.7	402.4	16.7	0.28	380.6	396.1	15.5	0.25
Peak 5 A	633	674.3	41.3	0.2	623.4	629.7	6.3	0.83	633.4	612.2	-21.3	0.48

Statistical significance is achieved in the early components of the cortical response. Latencies are attenuated in the IBS population compared to the controls over 3 days. The difference is always in the same direction, namely that the IBS CEP traces are more delayed, causing a right-shift in the CEP traces. This is contrary to what was expected- the hypothesis proposed that latencies would be reduced in IBS.

Figure 3.1.28 Peak 1 Amplitude

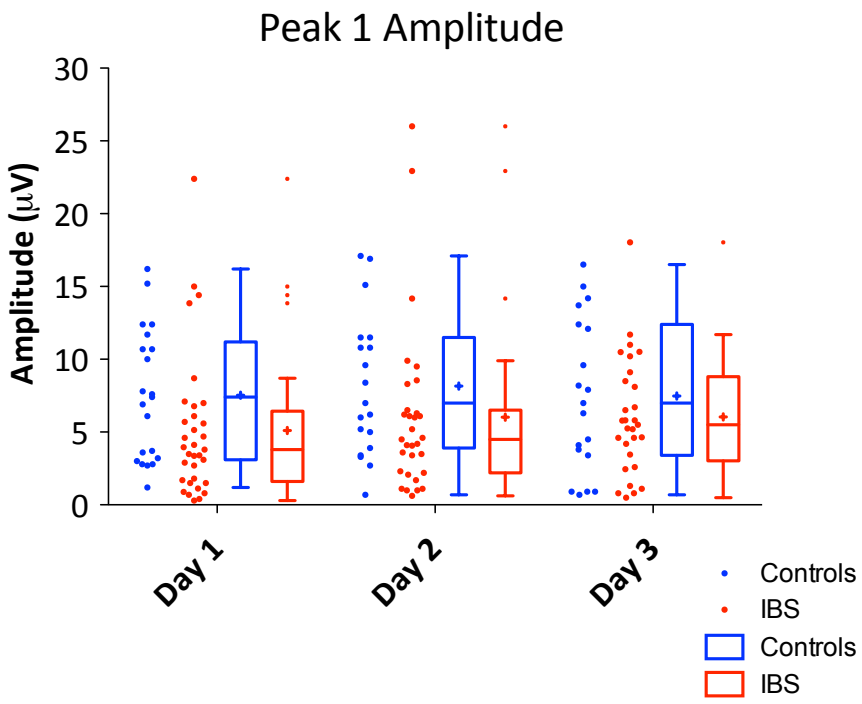


Table 3.1.28 Peak 1 Amplitude

Unpaired t test	Day 1	Day 2	Day 3
P value	0.0750	0.1864	0.2908
Mean ± SEM of Controls	7.529 ± 0.9821 N=21	8.163 ± 1.110 N=19	7.479 ± 1.210 N=19
Mean ± SEM of IBS	5.110 ± 0.8580 N=33	6.026 ± 1.045 N=31	6.043 ± 0.7472 N=29
Difference between means	2.419 ± 1.331	2.137 ± 1.595	1.436 ± 1.344
95% confidence interval	-0.2549 to 5.092	-1.072 to 5.346	-1.272 to 4.144

Figure 3.1.29 Peak 2 Amplitude

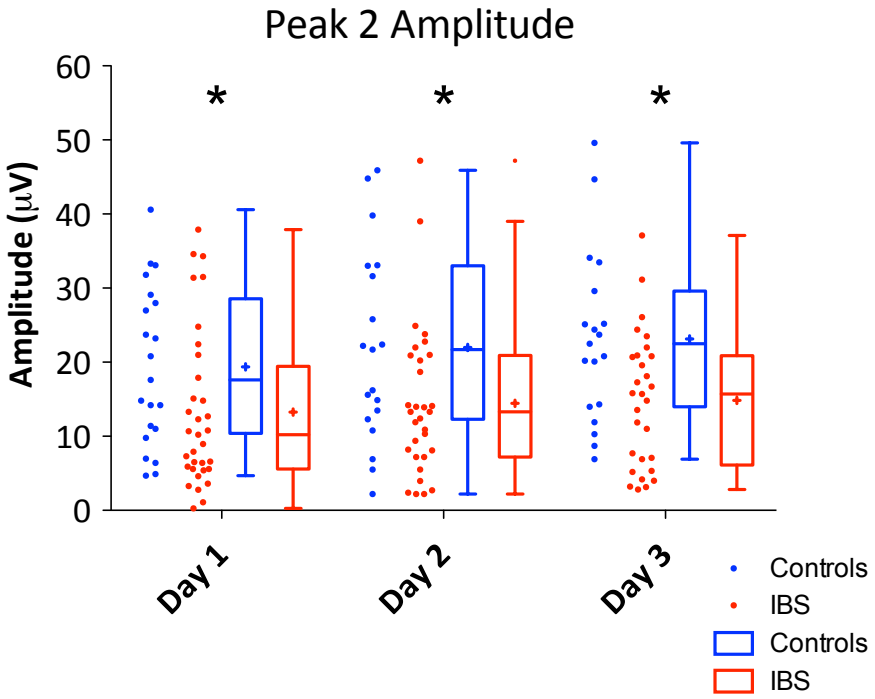


Table 3.1.29 Peak 2 Amplitude

Unpaired t test	Day 1	Day 2	Day 3
P value	0.0459	0.0272	0.0078
Mean ± SEM of Controls	19.36 ± 2.340 N=21	22.01 ± 2.995 N=19	23.14 ± 2.645 N=19
Mean ± SEM of IBS	13.26 ± 1.857 N=33	14.45 ± 1.844 N=31	14.85 ± 1.679 N=29
Difference between means	6.101 ± 2.984	7.563 ± 3.319	8.286 ± 2.977
95% confidence interval	0.1087 to 12.09	0.8829 to 14.24	2.287 to 14.28

Figure 3.1.30 Peak 3 Amplitude

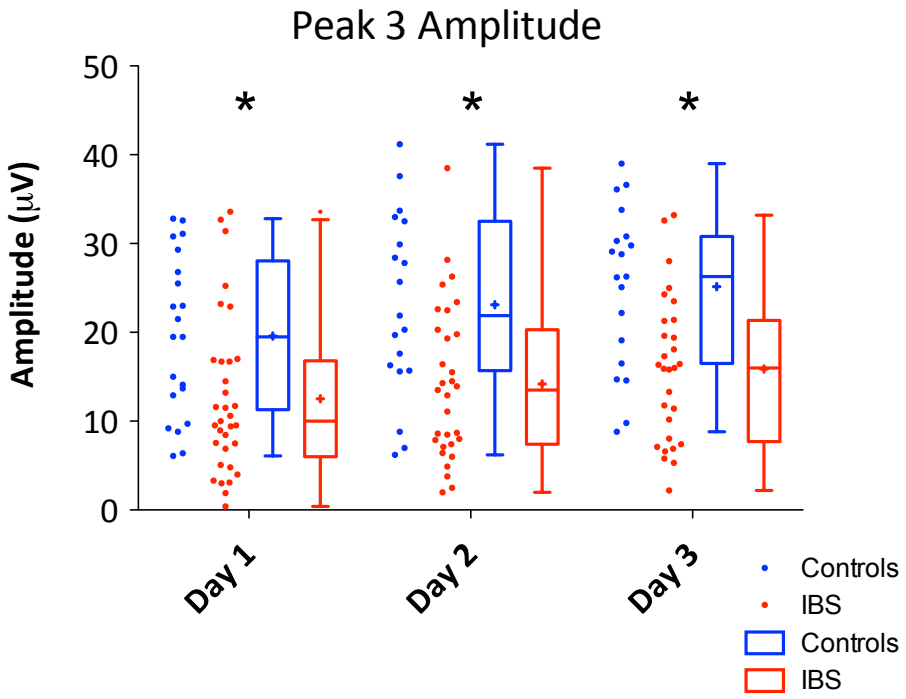


Table 3.1.30 Peak 3 Amplitude

Unpaired t test	Day 1	Day 2	Day 3
P value	0.0065	0.0019	0.0006
Mean ± SEM of Controls	19.58 ± 1.963 N=21	23.10 ± 2.355 N=19	25.14 ± 2.075 N=19
Mean ± SEM of IBS	12.51 ± 1.549 N=33	14.20 ± 1.563 N=31	15.87 ± 1.524 N=29
Difference between means	7.070 ± 2.494	8.901 ± 2.716	9.268 ± 2.522
95% confidence interval	2.061 to 12.08	3.436 to 14.37	4.187 to 14.35

Figure 3.1.31 Peak 4 Amplitude

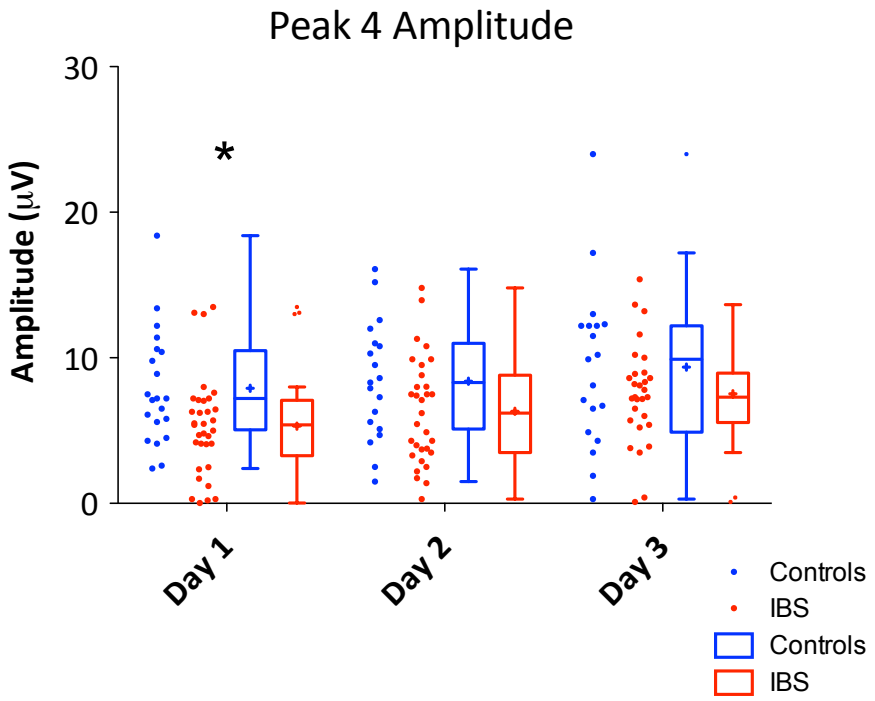


Table 3.1.31 Peak 4 Amplitude

Unpaired t test	Day 1	Day 2	Day 3
P value	0.0130	0.0689	0.1653
Mean ± SEM of Controls	7.905 ± 0.8499 N=21	8.395 ± 0.9238 N=19	9.368 ± 1.288 N=19
Mean ± SEM of IBS	5.310 ± 0.5959 N=33	6.336 ± 0.6559 N=31	7.525 ± 0.6428 N=29
Difference between means	2.595 ± 1.008	2.058 ± 1.106	1.843 ± 1.307
95% confidence interval	0.5699 to 4.620	-0.1680 to 4.285	-0.7905 to 4.477

Table 3.1.31b Summary of CEP Peak Amplitude Data

One Way ANOVA Comparison of Means: IBS vs. Controls

	Day 1				Day 2				Day 3			
(μV)	**Controls**	**IBS**	**Mean Δ**	**P-value**	**Controls**	**IBS**	**Mean Δ**	**P-value**	**Controls**	**IBS**	**Mean Δ**	**P-value**
Peak 1 A	7.5	5.1	-2.42	0.07	8.16	6.03	-2.14	0.18	7.5	6	-1.4	0.29
Peak 2 A	19.4	13.3	-6.1	0.045	22	14.4	-7.56	0.02	23.1	14.9	-8.3	0.007
Peak 3 A	19.58	12.51	-7.07	0.006	23.1	14.5	-8.9	0.001	25.1	15.9	-9.3	0.0006
Peak 4 A	7.9	5.3	-2.59	0.01	8.4	6.3	-2.1	0.068	9.4	7.5	-1.84	0.165

Again the data confounded the proposed hypothesis of rectal hypersensitivity. Group analysis of the CEP amplitudes show that IBS patients produced smaller amplitude traces compared to Controls, and that this was independent of the amount of electricity used (stimulus intensity on Visits 1 and 2 was not statistically different).

CORRELATIONS OF THE CEP STUDY DATA

Table 3.1.32 Spearman Correlation Coefficients of the IBS Group

Key

P- Psychoticism Scale of the EPQR

E- Extroversion Scale of the EPQR

N- Neuroticism scale of the EPQR

L- Lie Scale of the EPQR

A- Addiction Scale of the EPQR

C- Criminality Scale of the EPQR

VSI- Visceral Sensitivity Index

PHQ- Patient Health Questionnaire

Whiteley- Whiteley Index

HAD A- Anxiety scale of the HAD

HAD D- Depression scale of the HAD

SSTAI-S- State anxiety of the Spielberger State Trait Anxiety Inventory

SSTAI-T- Trait Anxiety of the Spielberger State Trait Anxiety Inventory

VDVASU- Unpleasantness scale of the Visual Descriptor Visual Analogue Scale

VDVASI- Intensity scale of the Visual Descriptor Visual Analogue Scale

ST- Sensory threshold

Spearman Correlation Coefficients

Prob > |r| under H0: Rho=0

	C	**SSTAI-T**							
P	0.37247	0.36715							
	0.0328	0.0356							
	A								
E	-0.41201								
	0.0172								
	C	**SSTAI-T**	**HAD A**	**HAD D**	**VDVASU**	**VDVAS I**	**PHQ**	**VSI**	**Whiteley**
N	0.61553	0.5835	0.76783	0.34422	0.54057	0.44507	0.55436	0.48272	0.49252
	0.0001	0.0004	<.0001	0.0498	0.0012	0.0094	0.0008	0.0044	0.0036
	A	**C**							
L	-0.6453	-0.0678							
	<.0001	0.7077							
	E	**L**	**PHQ**						
A	-0.41201	-0.6453	0.37629						
	0.0172	<.0001	0.0309						
	N	**L**	**P**	**HAD A**	**PHQ**	**Whiteley**			
C	0.61553	-0.0678	0.37247	0.52365	0.45061	0.43997			
	0.0001	0.7077	0.0328	0.0018	0.0085	0.0104			
	N	**SSTAI-T**	**HAD A**	**HAD D**	**VDVASU**	**VDVAS I**	**PHQ**	**Whiteley**	**ST**
VSI	0.48272	0.39704	0.37085	0.42135	0.48114	0.50155	0.46713	0.55978	0.38714
	0.0044	0.0221	0.0336	0.0146	0.0046	0.0029	0.0061	0.0007	0.026

	N	A	C	SSTAI-S	SSTAI-T	HAD A	HAD D	VDVASU	VDVAS I	VSI	Whiteley
PHQ	0.55436	0.37629	0.45061	0.34836	0.67089	0.38868	0.66543	0.56999	0.67615	0.46713	0.50269
	0.0008	0.0309	0.0085	0.0469	<.0001	0.0254	<.0001	0.0005	<.0001	0.0061	0.0029

	N	C	SSTAI-T	HAD A	HAD D	VDVASU	VDVAS I	PHQ	VSI
Whiteley	0.49252	0.43997	0.44766	0.53542	0.56976	0.49318	0.44376	0.50269	0.55978
	0.0036	0.0104	0.009	0.0013	0.0005	0.0035	0.0097	0.0029	0.0007

	N	SSTAI-S	SSTAI-T	C	VDVASU	PHQ	VSI	Whiteley
HAD A	0.76783	0.36388	0.57662	0.52365	0.34935	0.38868	0.37085	0.53542
	<.0001	0.0374	0.0004	0.0018	0.0463	0.0254	0.0336	0.0013

	N	SSTAI-T	VDVASU	VDVAS I	PHQ	VSI	Whiteley
HAD D	0.34422	0.62891	0.57476	0.56411	0.66543	0.42135	0.56976
	0.0498	<.0001	0.0005	0.0006	<.0001	0.0146	0.0005

	SSTAI-T	HAD A	PHQ
SSTAI-S	0.57193	0.36388	0.34836
	0.0005	0.0374	0.0469

	N	SSTAI-S	P	HAD A	HAD D	VDVASU	VDVAS I	PHQ	VSI	Whiteley
SSTAI-T	0.5835	0.57193	0.36715	0.57662	0.62891	0.54086	0.38273	0.67089	0.39704	0.44766
	0.0004	0.0005	0.0356	0.0004	<.0001	0.0012	0.0279	<.0001	0.0221	0.009

	N	SSTAI-T	HAD A	HAD D	VDVASI	PHQ	VSI	Whiteley
VDVAU	0.54057	0.54086	0.34935	0.57476	0.78928	0.56999	0.48114	0.49318
	0.0012	0.0012	0.0463	0.0005	<.0001	0.0005	0.0046	0.0035

	N	SSTAI-T	HAD D	VDVASU	PHQ	VSI	Whiteley
VDVASI	0.44507	0.38273	0.56411	0.78928	0.67615	0.50155	0.44376
	0.0094	0.0279	0.0006	<.0001	<.0001	0.0029	0.0097

	VSI
ST	0.38714
	0.026

CHAPTER THREE, SECTION TWO:

CYTOKINE STUDY RESULTS

The patients assembled for the blood test were drawn from the initial group of IBS patients who had the CEP testing. There was only one IBS patient who was the exception to this, preferring just to give blood and not go through with CEP testing. Similarly, a subset of controls (9 in total) emerged from the parent CEP group for blood testing.

Table 3.2.1 IBS/Control Demographics

	Controls	**IBS**
Sex	1 Males, 8 Females	4 Males, 21 Females
Mean age	36.4	40.1
Median age	37	41
Mode age	n/a	28
Age range	25 to 46	18 to 64

Figure 3.2.1 IBS subtypes for the Cytokine study

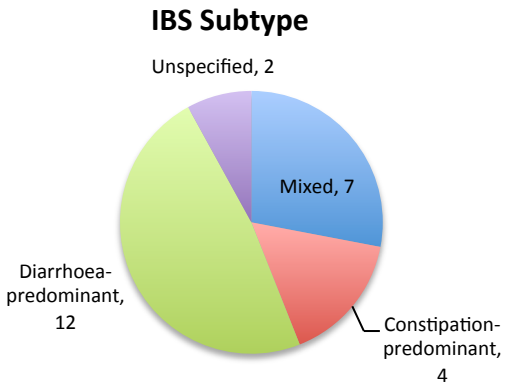


Figure 3.2.2 Irritable Bowel Severity Scoring System

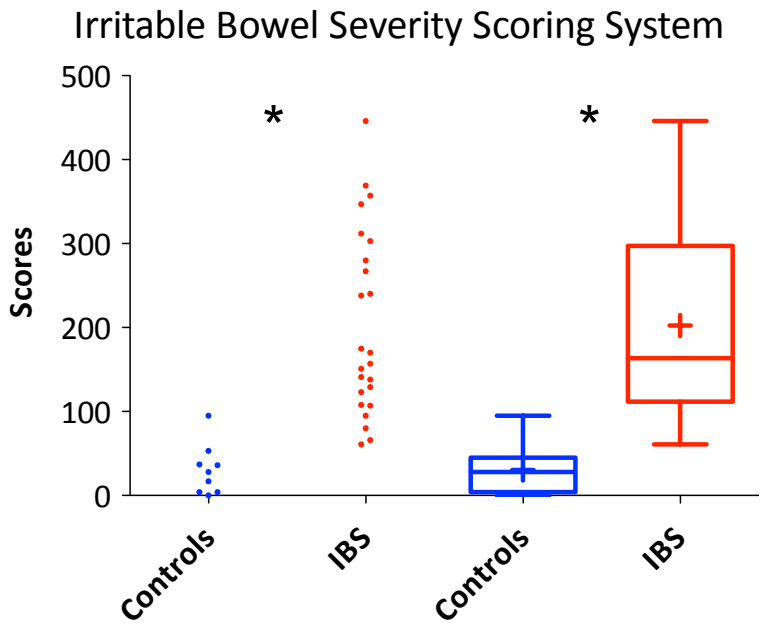


Table 3.2.2 Irritable Bowel Severity Scoring System

Unpaired t test	
P value	< 0.0001
Mean ± SEM of Controls	30 ± 10 N=9
Mean ± SEM of IBS	203 ± 22 N=24
Difference between means	-172 ± 37
95% confidence interval	-248 to -96

Scoring

IBS in remission: <75; Mild IBS: 75 to 175; Moderate IBS: 175 to 300; Severe IBS: >300 ; (Maximum score of 500)

Analysis

The Irritable Bowel Severity Scoring System was a late addition to the study protocol. Its inclusion was brought about because of the need for a quantitative marker of IBS clinical severity. It assesses symptoms in the preceding 10 days. According to the scoring system the IBS mean value of 202.5 fell within the category of moderate IBS. Two of the 25 IBS patients fell within the category of being in clinical remission, scoring less than 75. The range of severity was broad with values recorded between 61 and 446.

Figure 3.2.3 Irritable Bowel Syndrome Impact Scale

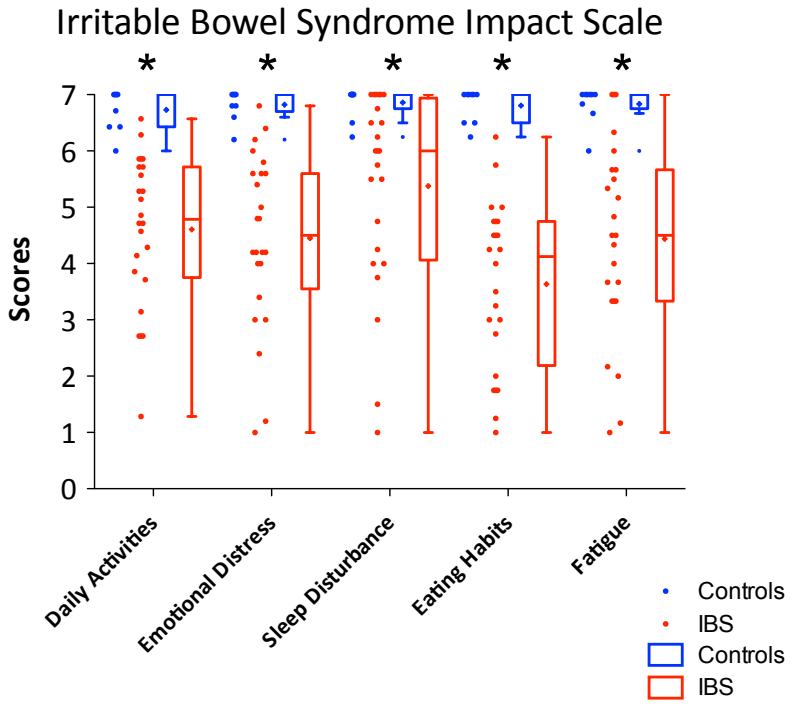


Table 3.2.3 Irritable Bowel Syndrome Impact Scale

Unpaired t test	Daily Activities	Emotional Distress	Sleep Disturbance
P value	< 0.0001	< 0.0001	0.0173
Mean ± SEM of Controls	6.730 ± 0.1224 N=9	6.822 ± 0.09095 N=9	6.861 ± 0.09420 N=9
Mean ± SEM of IBS	4.607 ± 0.2747 N=24	4.450 ± 0.3147 N=24	5.375 ± 0.3567 N=24
Difference between means	2.123 ± 0.4589	2.372 ± 0.5218	1.486 ± 0.5911
95% confidence interval	1.187 to 3.059	1.308 to 3.437	0.2804 to 2.692

Unpaired t test	Eating Habits	Fatigue
P value	< 0.0001	0.0003
Mean ± SEM of Controls	6.806 ± 0.1002 N=9	6.833 ± 0.1111 N=9
Mean ± SEM of IBS	3.635 ± 0.2998 N=24	4.438 ± 0.3562 N=24
Difference between means	3.170 ± 0.4981	2.396 ± 0.5913
95% confidence interval	2.154 to 4.186	1.190 to 3.602

Analysis

Interpretation of the results is based on the understanding that the maximum score per category is 7, and that this score indicates normality (see Figure 1.3.2) Deflections towards the value of zero are associated with increasing symptom severity. The IBS-IS is useful because it gives an idea of impaired quality of life, disruption with daily affairs and food intolerance.

The IBS-IS delivers statistical significance across all domains. The graphic below is taken from the paper that first published the IBS-IS. It shows the relative scoring of varying IBS severity.

CYTOKINE ANALYSIS

The following section features the cytokine data. Each cytokine is presented individually. 6 point dose-response curves show the amount of cytokine produced following the administration of E.coli LPS. The initial point on each graph is an unstimulated sample, i.e. LPS concentration of zero. The cytokines are separated into the two groups according to their pro and anti-inflammatory roles.

Figure 3.2.4 TNF-α release from LPS-stimulated PBMCs

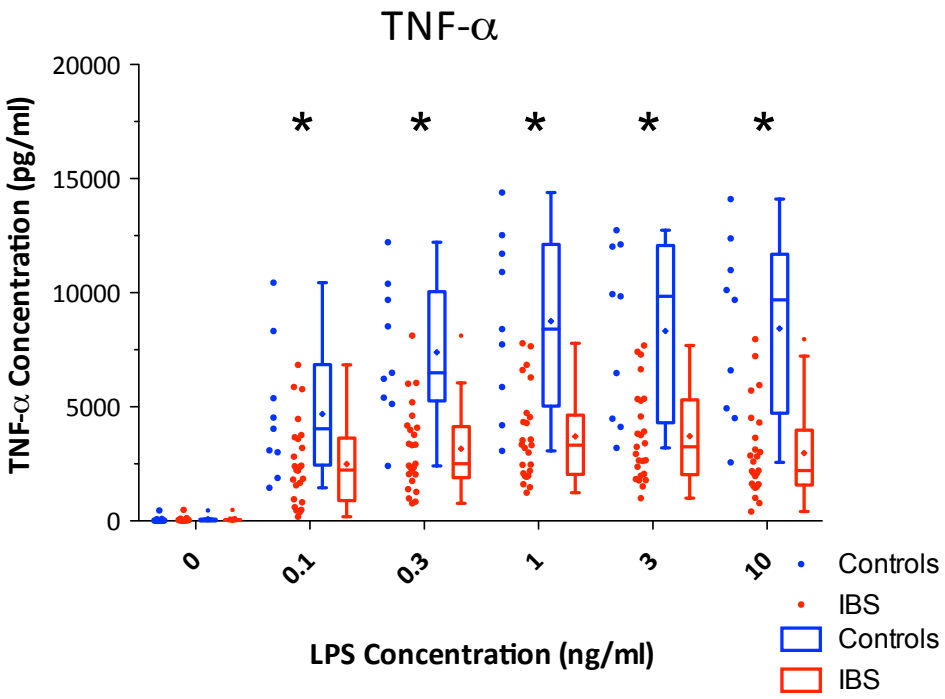


Table 3.2.4 TNF-α release from LPS-stimulated PBMCs- One Way ANOVA

Comparison of Means

LPS Concentration (ng/ml)	IBS Mean	Control Mean	Difference Of The Means	95% CI	P Value
0	57.93	77.84	-19.91	(-105.73, 65.91)	0.6398
0.1	2496.46	4683.95	-2187.5	(-3901.27, -473.72)	0.014
0.3	3161.3	7389.24	-4227.94	(-5973.10, -2482.77)	<0.0001
1	3701.43	8758.32	-5056.88	(-7110.81, -3002.96)	<0.0001
3	3718.72	8328.48	-4609.76	(-6634.06, -2585.47)	<0.0001
10	2970.33	8433.07	-5462.75	(-7526.47, -3399.03)	<0.0001

Analysis

Figure 3.2.4 demonstrates a consistent grouping of TNF-Alpha expression in the IBS group. Notably there is no separation between the control and IBS groups in the unstimulated samples. The Control group produces significantly more TNF-Alpha during LPS stimulation. However there is a larger range in the control group compared to the IBS group.

Figure 3.2.5 IFN-γ release from LPS-stimulated PBMCs

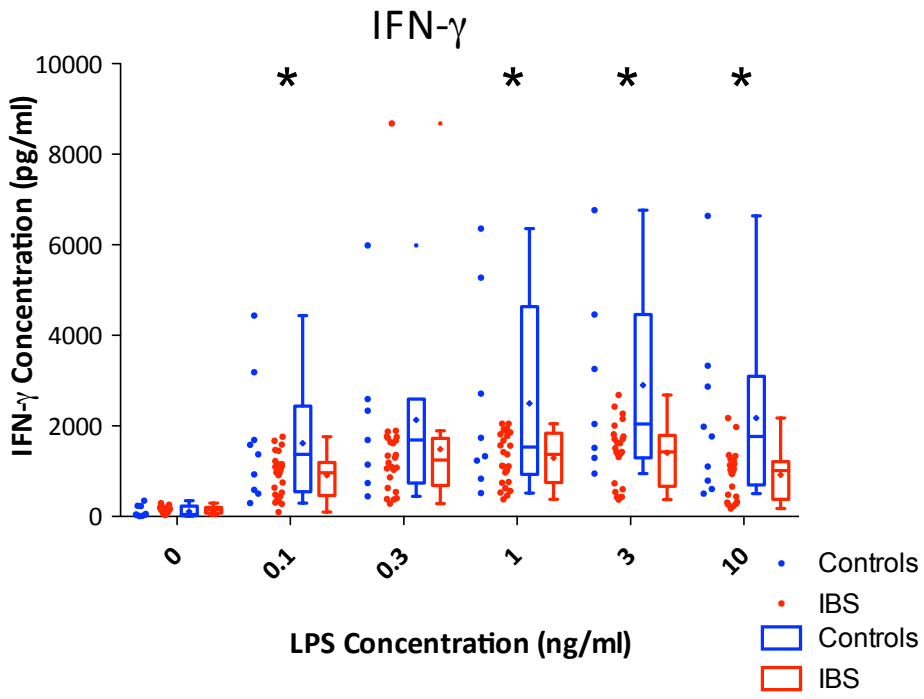


Table 3.2.5 IFN-γ release from LPS-stimulated PBMCs- - One Way ANOVA

Comparison of Means

LPS Concentration (ng/ml)	IBS Mean	Control Mean	Difference Of The Means	95% CI	P Value
0	137.61	111.71	25.9	(-43.60, 95.40)	0.4534
0.1	910.52	1620.96	-710.44	(-1342.46, -78.42)	0.0288
0.3	1484.17	2134.29	-650.11	(-2121.53, 821.30)	0.3736
1	1293.04	2501	-1207.96	(-2152.62, -263.29)	0.0139
3	1406.77	2897.64	-1490.88	(-2464.42, -517.33)	0.0039
10	921.22	2177.73	-1256.5	(-2105.76, -407.25)	0.005

Analysis

Statistical significance is reached at various LPS concentrations (0.1, 1, 3 and 10ng/ml). However, the scattergrams clearly identify Control outliers which skew the data. Removal of these outliers obviates the statistical difference between the two groups.

Figure 3.2.6 IL-1-β release from LPS-stimulated PBMCs

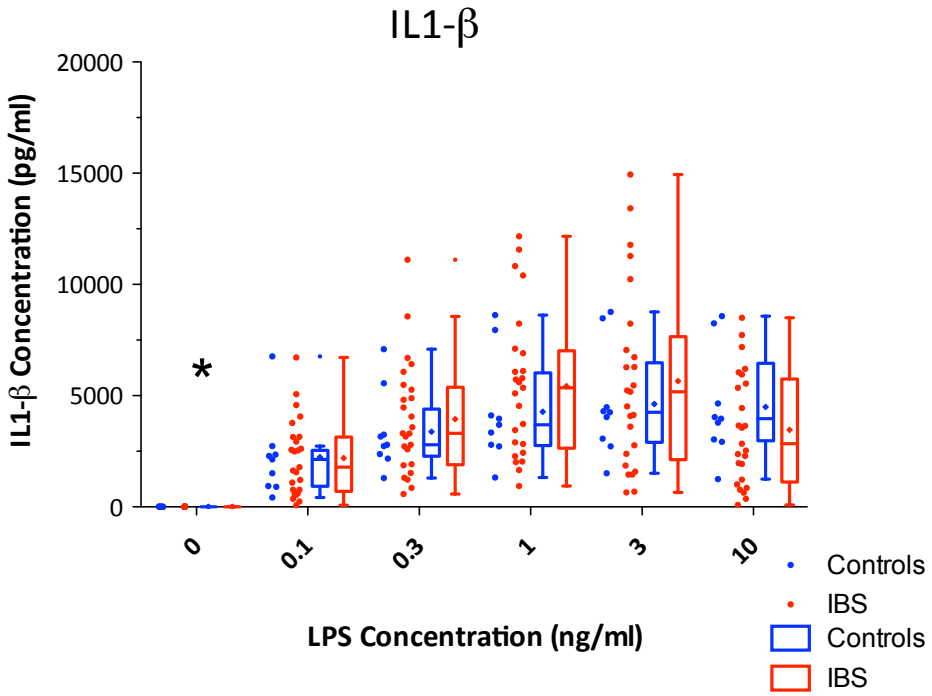


Table 3.2.6 IL-1-β release from LPS-stimulated PBMCs- One Way ANOVA

Comparison of Means

LPS Concentration (ng/ml)	IBS Mean	Control Mean	Difference Of The Means	95% CI	P Value
0	9.4	4.18	5.22	(0.23, 10.20)	0.0408
0.1	2195.63	2229.82	-34.18	(-1419.87, 1351.51)	0.9602
0.3	3945.87	3383.88	561.99	(-1310.01, 2433.99)	0.5452
1	5433.18	4279.81	1153.37	(-1253.15, 3559.90)	0.3363
3	5653.68	4625.97	1027.72	(-1913.85, 3969.28)	0.4818
10	3469.87	4497.45	-1027.58	(-2988.26, 933.10)	0.2937

Analysis

IL-1β expression is not shown to be different in the LPS-stimulated samples between the two groups.

Figure 3.2.7 IL-2 release from LPS-stimulated PBMCs

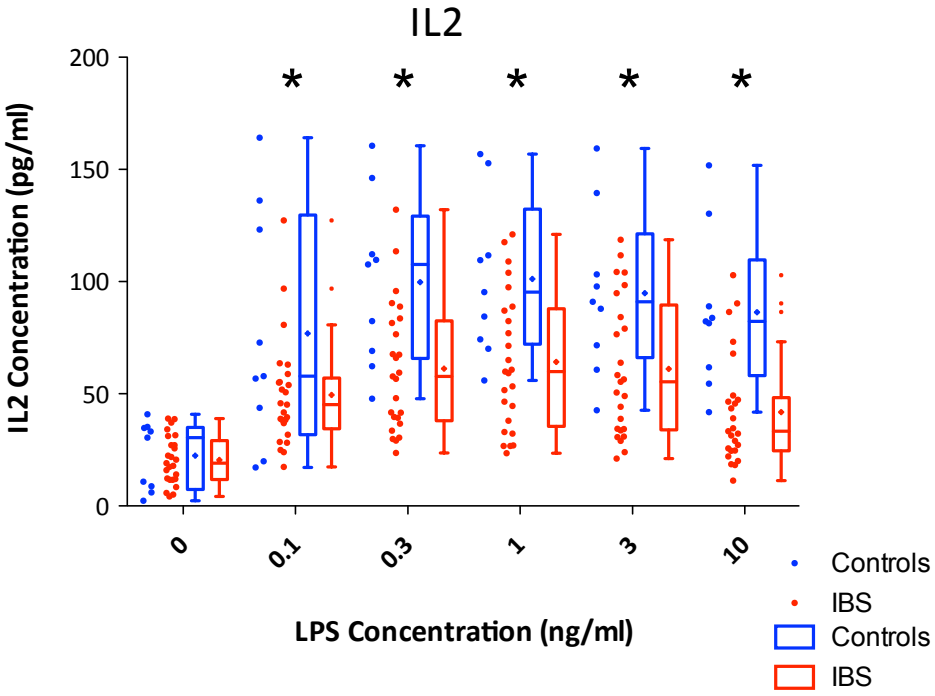


Table 3.2.7 IL-2 release from LPS-stimulated PBMCs- One Way ANOVA

Comparison of Means

LPS Concentration (ng/ml)	IBS Mean	Control Mean	Difference Of The Means	95% CI	P Value
0	20.5	22.5	-2	(-11.46, 7.45)	0.6688
0.1	49.47	76.87	-27.4	(-53.95, -0.84)	0.0436
0.3	61.24	99.79	-38.54	(-63.18, -13.90)	0.0032
1	64.16	101.22	-37.06	(-62.10, -12.01)	0.005
3	61.06	94.88	-33.82	(-59.27, -8.37)	0.0108
10	41.81	86.31	-44.51	(-66.27, -22.74)	0.0002

Analysis

IL-2 release is statistically different between the two groups in the LPS-stimulated samples. The IBS group demonstrates a much tighter range of cytokine expression compared to the IBS group. A few control subjects express large concentrations of IL-2. They may represent outliers, or be part of the normal distribution.

Figure 3.2.8 IL-6 release from LPS-stimulated PBMCs

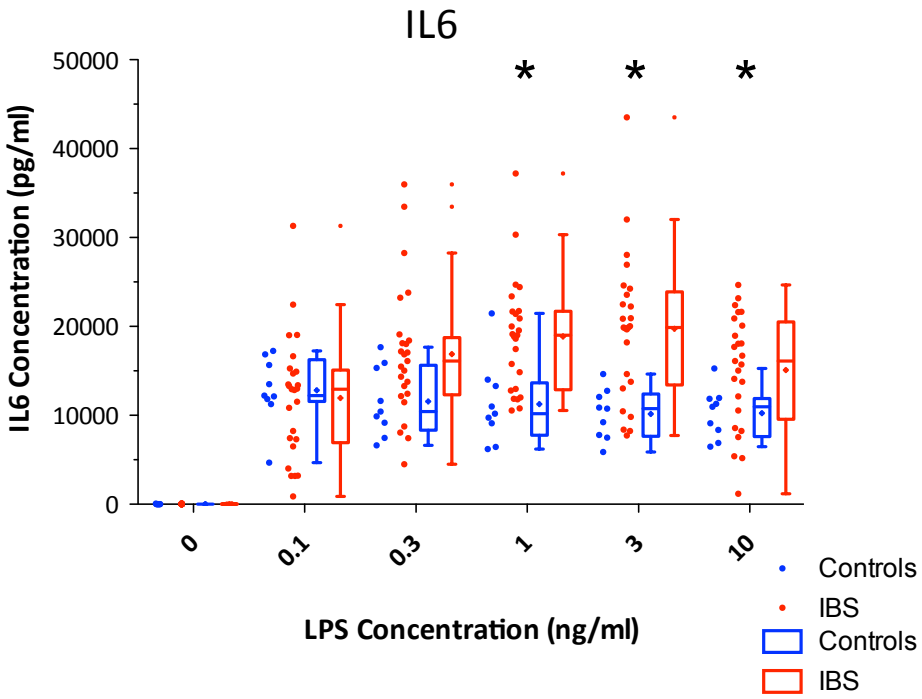


Table 3.2.8 IL-6 release from LPS-stimulated PBMCs- One Way ANOVA

Comparison of Means

LPS Concentration (ng/ml)	IBS Mean	Control Mean	Difference Of The Means	95% CI	P Value
0	29.39	16.81	12.57	(-4.93, 30.07)	0.1531
0.1	11946.56	12823.69	-877.12	(-5847.41, 4093.17)	0.7216
0.3	16898.62	11573.36	5325.26	(-63.55, 10714.07)	0.0526
1	18875.22	11269.47	7605.74	(2890.51, 12320.98)	0.0025
3	19723.86	10167.8	9556.06	(3829.76, 15282.36)	0.0018
10	15105.89	10242.13	4863.76	(383.76, 9343.75)	0.0343

Analysis

This data demonstrates good separation between the two groups for LPS-stimulated IL-6 expression. This becomes more marked with higher concentrations of LPS, reaching statistical significance at concentrations greater than 0.3ng/ml.

Figure 3.2.9 IL-8 release from LPS-stimulated PBMCs

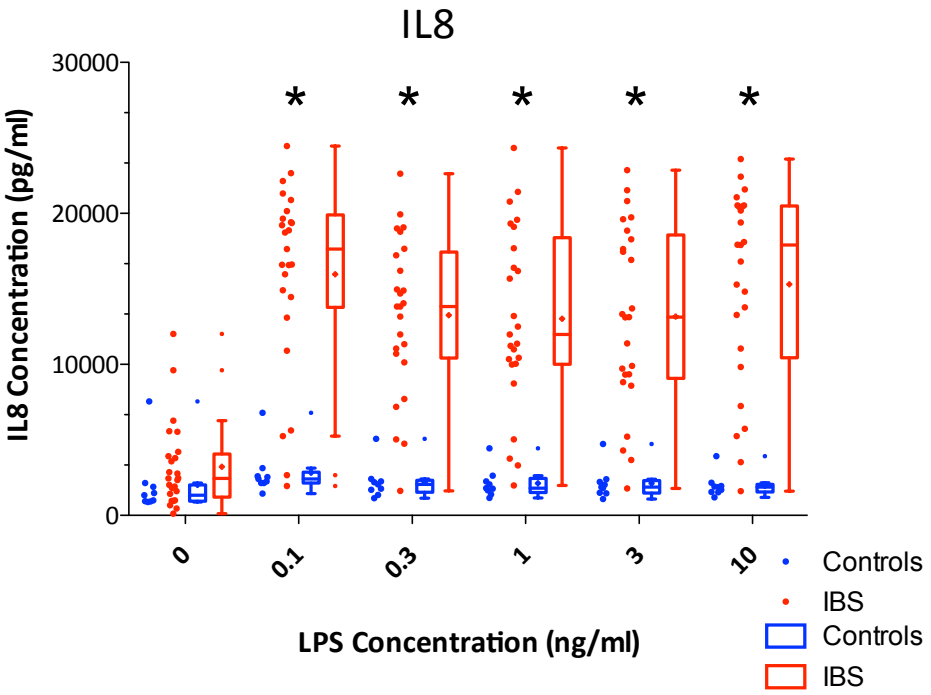


Table 3.2.9 IL-8 release from LPS-stimulated PBMCs- One Way ANOVA

Comparison of Means

LPS Concentration (ng/ml)	IBS Mean	Control Mean	Difference Of The Means	95% CI	P Value
0	3215.21	2019.75	1195.46	(-920.98, 3311.89)	0.2584
0.1	15966.99	2829.47	13137.52	(8834.91, 17440.13)	<0.0001
0.3	13259.12	2217.8	11041.32	(7438.46, 14644.18)	<0.0001
1	13017.76	2129.75	10888.01	(6774.28, 15001.74)	<0.0001
3	13165.73	2126	11039.73	(6878.61, 15200.84)	<0.0001
10	15296.58	1979.57	13317.01	(8873.03, 17761.00)	<0.0001

Analysis

Figure 3.2.9 shows marked differences in IL-8 expression between the two groups.

Figure 3.2.10 IL-12 release from LPS-stimulated PBMCs

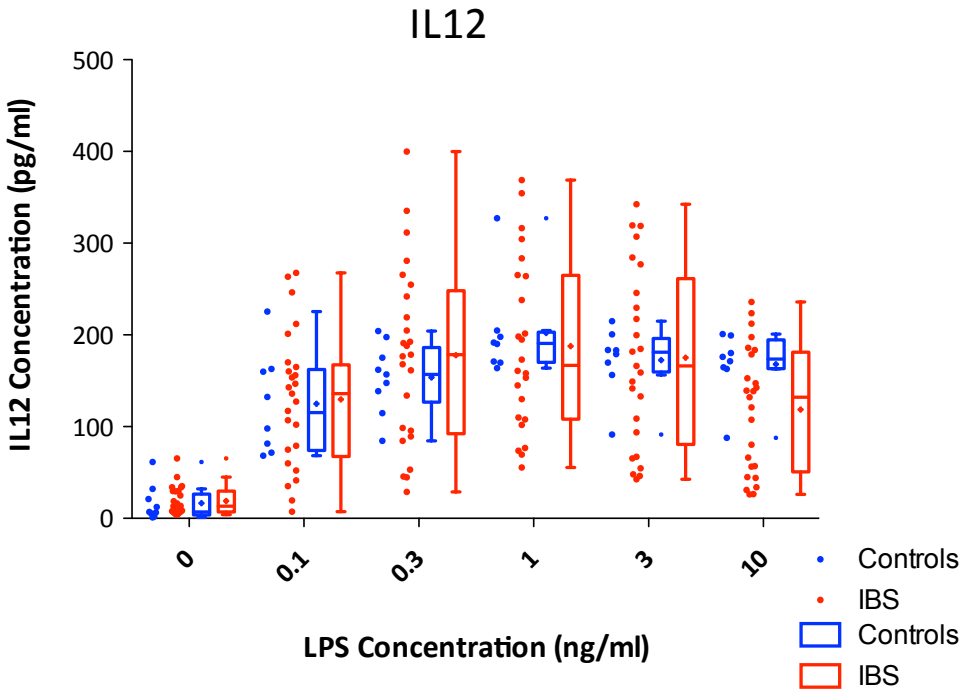


Table 3.2.10 IL-12 release from LPS-stimulated PBMCs- One Way ANOVA

Comparison of Means

LPS Concentration (ng/ml)	IBS Mean	Control Mean	Difference Of The Means	95% CI	P Value
0	18.99	16.57	2.42	(-10.73, 15.57)	0.7101
0.1	129.78	125.05	4.73	(-53.13, 62.60)	0.8686
0.3	177.76	153.5	24.26	(-44.26, 92.78)	0.476
1	187.71	202.02	-14.32	(-85.51, 56.88)	0.6842
3	175.33	172.38	2.95	(-68.84, 74.74)	0.9337
10	118.62	167.93	-49.31	(-100.72, 2.09)	0.0595

Analysis

IL-12 expression in IBS patients produces a broad range of data. This is in contrast to the tight grouping of results in the control group. Statistical significance is not reached

Figure 3.2.11 IL-4 release from LPS-stimulated PBMCs

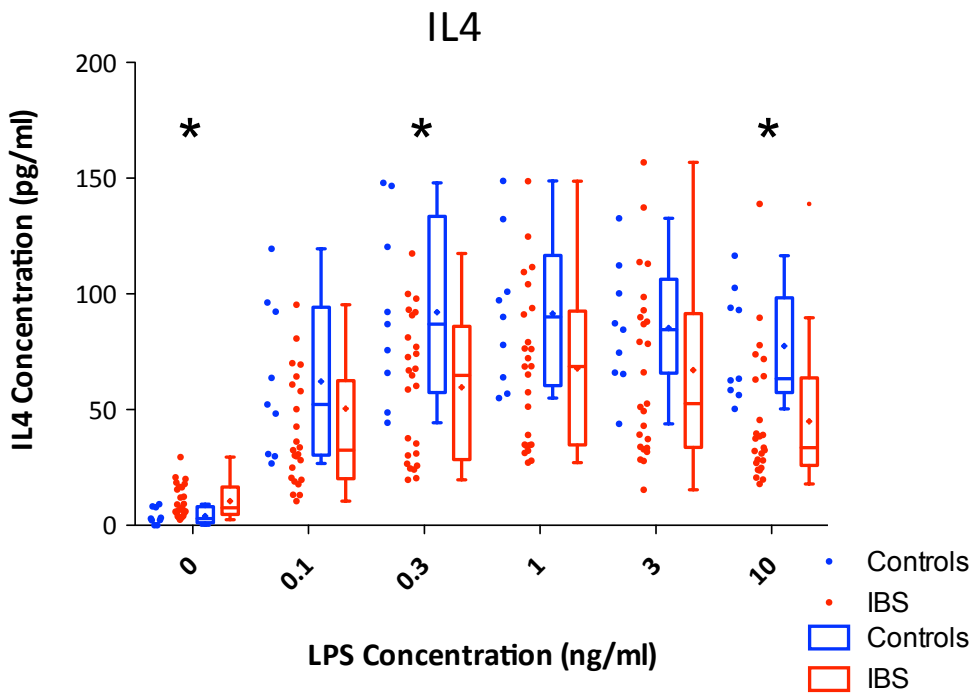


Table 3.2.11 IL-4 release from LPS-stimulated PBMCs- One Way ANOVA

Comparison of Means

LPS Concentration (ng/ml)	IBS Mean	Control Mean	Difference Of The Means	95% CI	P Value
0	10.49	4	6.5	(1.42, 11.57)	0.0137
0.1	50.48	62.19	-11.71	(-54.16, 30.73)	0.578
0.3	59.63	92.1	-32.47	(-58.10, -6.84)	0.0147
1	67.82	91.48	-23.65	(-50.31, 3.00)	0.0801
3	67.11	85.23	-18.12	(-46.03, 9.79)	0.1955
10	44.95	77.47	-32.51	(-54.08, -10.95)	0.0043

Analysis

Table 3.2.11 suggests a statistical separation of the two groups, but the scattergram indicates that both groups produce broadly similar data.

Figure 3.2.12 IL-5 release from LPS-stimulated PBMCs

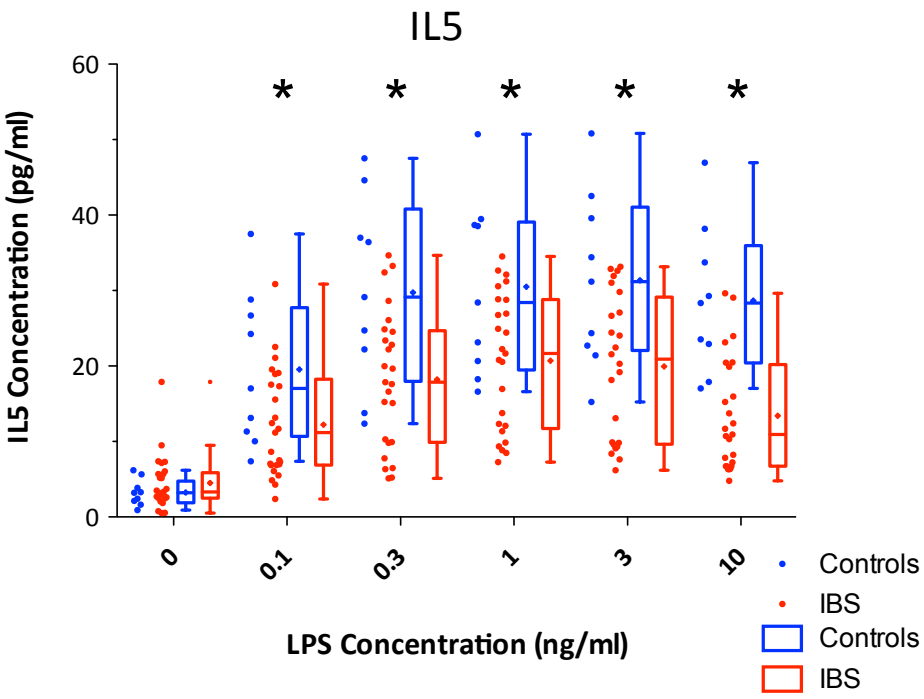


Table 3.2.12 IL-5 release from LPS-stimulated PBMCs- One Way ANOVA

Comparison of Means

LPS Concentration (ng/ml)	IBS Mean	Control Mean	Difference Of The Means	95% CI	P Value
0	4.5	3.25	1.25	(-1.34, 3.83)	0.3337
0.1	12.21	19.57	-7.35	(-13.69, -1.02)	0.0243
0.3	18.24	29.75	-11.52	(-19.50, -3.54)	0.006
1	20.69	30.5	-9.81	(-17.53, -2.09)	0.0144
3	19.93	31.37	-11.44	(-19.46, -3.41)	0.0067
10	13.4	28.66	-15.26	(-21.68, -8.83)	<0.0001

Analysis

LPS-stimulated PBMCs in the control group produced more IL-5 than in the IBS group.

Figure 3.2.13 IL-10 release from LPS-stimulated PBMCs

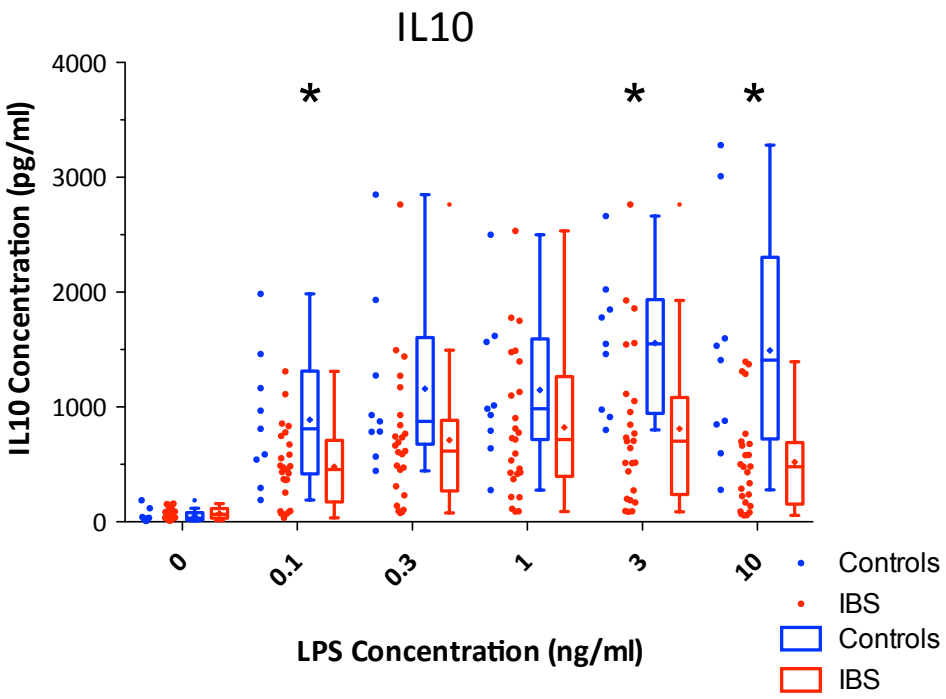


Table 3.2.13 IL-10 release from LPS-stimulated PBMCs- One Way ANOVA

Comparison of Means

LPS Concentration (ng/ml)	IBS Mean	Control Mean	Difference Of The Means	95% CI	P Value
0	73.8	53.97	19.83	(-21.37, 61.02)	0.3342
0.1	481.03	889.41	-408.38	(-731.18, -85.59)	0.0148
0.3	711.5	1160.58	-449.08	(-957.09, 58.94)	0.0812
1	822.35	1146.91	-324.56	(-825.83, 176.72)	0.1966
3	810.31	1557.42	-747.11	(-1267.27, -226.94)	0.0063
10	519.55	1492.97	-973.42	(-1477.83, -469.00)	0.0004

Analysis

Figure 3.2.13 shows equivalent IL-10 release in the two groups despite the p values which suggest otherwise.

Figure 3.2.14 IL-13 release from LPS-stimulated PBMCs

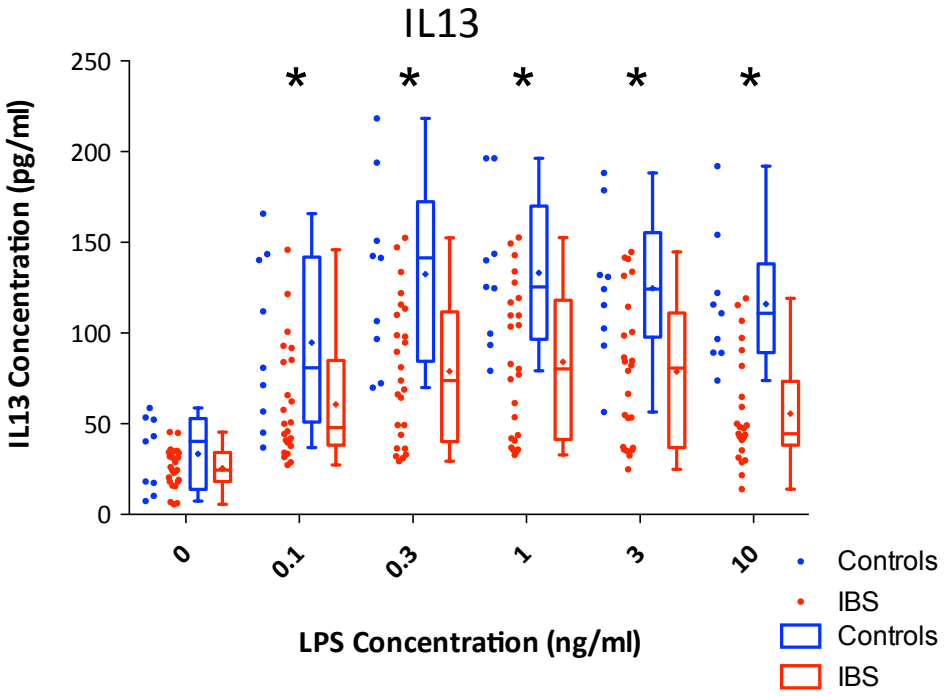


Table 3.2.14 IL-13 release from LPS-stimulated PBMCs- One Way ANOVA

Comparison of Means

LPS Concentration (ng/ml)	IBS Mean	Control Mean	Difference Of The Means	95% CI	P Value
0	25.44	33.47	-8.03	(-18.93, 2.87)	0.1434
0.1	60.63	94.71	-34.08	(-63.01, -5.16)	0.0224
0.3	78.85	132.53	-53.68	(-87.05, -20.30)	0.0025
1	84.11	133.22	-49.11	(-81.64, -16.57)	0.0043
3	78.72	124.68	-45.96	(-77.89, -14.03)	0.0062
10	55.65	115.98	-60.33	(-85.16, -35.50)	<0.0001

Analysis

IL-13 release is consistently and significantly increased in control subjects following LPS-stimulation.

CORRELATIONS OF THE CYTOKINE DATA

Table 3.2.15 Spearman Correlation Coefficients of the IBS Group

Key

P- Psychoticism Scale of the EPQR

A- Addiction Scale of the EPQR

C- Criminality Scale of the EPQR

VSI- Visceral Sensitivity Index

HAD A- Anxiety scale of the HAD

HAD D- Depression scale of the HAD

SSTAI-S- State anxiety of the Spielberger State Trait Anxiety Inventory

SSTAI-T- Trait Anxiety of the Spielberger State Trait Anxiety Inventory

VDVASU- Unpleasantness scale of the Visual Descriptor Visual Analogue Scale

VDVASI- Intensity scale of the Visual Descriptor Visual Analogue Scale

IBSSS- Irritable Bowel Severity Scoring System

IBSIS DA- Daily Activities scale

IBSIS ED- Emotional Distress scale

IBSIS EH- Eating Habits scale

IBSIS SD- Sleeping Dysfunction

IBSIS F- Fatigue Scale

Spearman Correlation Coefficients

Prob > |r| under H0: Rho=0

	IL6	TNF α
P	-0.43417	-0.50002
	0.034	0.0128

	IL13	IL2
A	-0.45475	-0.46009
	0.0256	0.0237

	IL8
C	-0.41465
	0.0439

	IFN γ
VSI	0.45551
	0.0253

	SSTAI-S	SSTAI-T	HAD D	IFN γ	IL10
HAD A	0.48486	0.68163	0.55896	0.55433	0.40744
	0.019	0.0003	0.0056	0.0049	0.0481

	SSTAI-S	SSTAI-T	HAD A	TNF α	IL 13
HAD D	0.67264	0.64604	0.55896	-0.44428	-0.43215
	0.0004	0.0009	0.0056	0.0337	0.035

	SSTAI-T	HAD A	HAD D	VDVAS U	TNF α
SSTAI-S	0.83313	0.48486	0.67264	0.42756	-0.42189

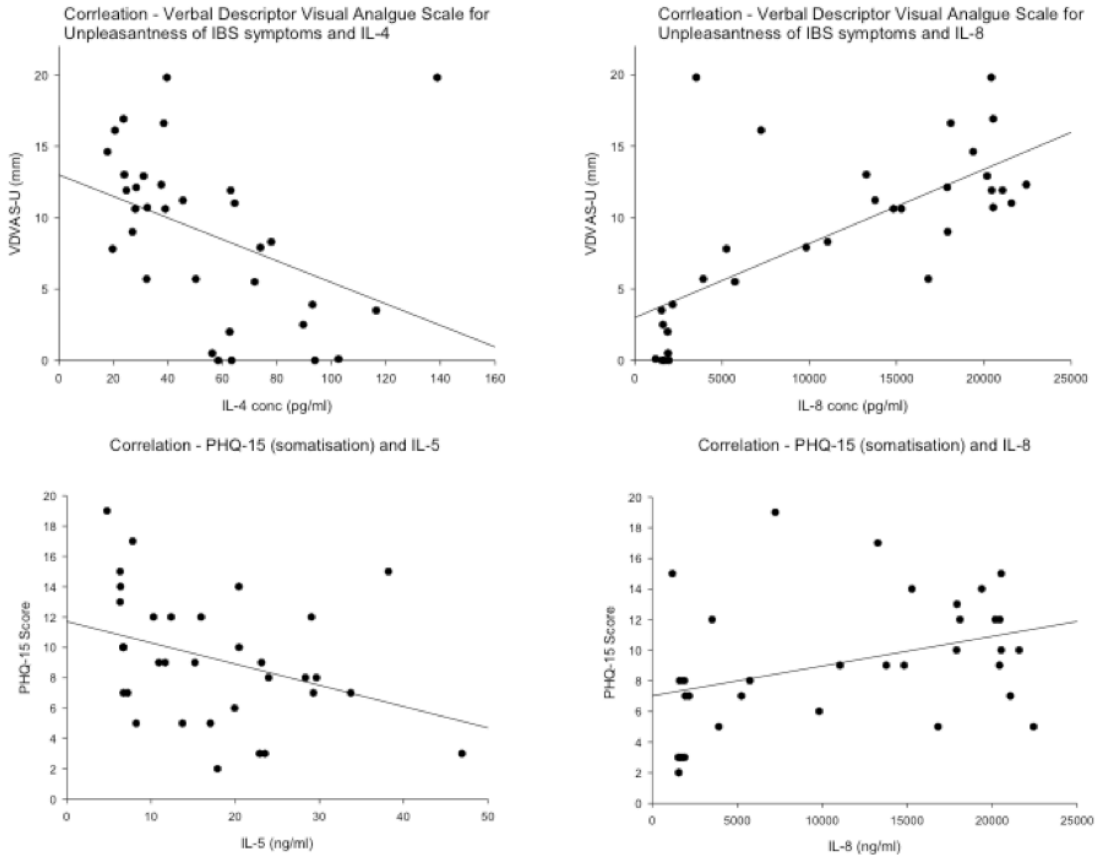
	<.0001	0.019	0.0004	0.0472	0.0449		
	SSTAI-S	**HAD A**	**HAD D**	**VDVAS U**	**IBSIS ED**	**IBSIS SD**	
SSTAI-T	0.83313	0.68163	0.64604	0.54641	-0.4434	-0.4222	
	<.0001	0.0003	0.0009	0.0085	0.0341	0.0448	
	IBSSS	**SSTAI-S**	**SSTAI-T**	**VDVAS I**	**IBSIS DA**	**IBSIS ED**	**IBSIS F**
VDVAS U	0.5661	0.42756	0.54641	0.82881	-0.56346	-0.47775	-0.42627
	0.006	0.0472	0.0085	<.0001	0.0063	0.0245	0.0479
	IBSSS	**VDVAS U**	**IBSIS DA**	**IBSIS F**	**IL5**	**IL13**	**IL2**
VDVAS I	0.60248	0.82881	-0.47509	-0.43168	-0.41159	-0.44285	-0.42064
	0.003	<.0001	0.0255	0.0448	0.0457	0.0302	0.0407
	VDVAS U	**VDVAS I**	**IBSIS DA**	**IBSIS ED**	**IBSIS SD**	**IBSIS F**	
IBSSS	0.5661	0.60248	-0.58594	-0.56371	-0.53981	-0.43801	
	0.006	0.003	0.0033	0.0051	0.0078	0.0366	
	IBSSS	**VDVAS U**	**VDVAS I**	**IBSIS ED**	**IBSIS F**		
IBSIS DA	-0.58594	-0.56346	-0.47509	0.87028	0.76681		
	0.0033	0.0063	0.0255	<.0001	<.0001		
	IBSSS	**SSTAI-T**	**IBSIS DA**	**IBSIS F**			
IBSIS ED	-0.56371	-0.4434	0.87028	0.82245			
	0.0051	0.0341	<.0001	<.0001			
	IBSIS DA	**IBSIS F**					
IBSIS EH	0.49578	0.41917					
	0.0161	0.0465					
	IBSSS	**SSTAI-T**	**IBSIS F**				
IBSIS SD	-0.53981	-0.4222	0.4248				
	0.0078	0.0448	0.0433				
	IBSSS	**VDVAS U**	**VDVAS I**	**IBSIS DA**	**IBSIS ED**	**IBSIS SD**	**IBSIS EH**
IBSIS F	-0.43801	-0.42627	-0.43168	0.76681	0.82245	0.4248	0.41917
	0.0366	0.0479	0.0448	<.0001	<.0001	0.0433	0.0465
	SSTAI-S	**HAD D**	**P**				
TNF α	-0.42189	-0.44428	-0.50002				
	0.0449	0.0337	0.0128				
	HAD A	**VSI**					
IFN γ	0.55433	0.45551					
	0.0049	0.0253					
	A	**VDVAS I**					
IL2	-0.46009	-0.42064					
	0.0237	0.0407					
	P						
IL6	-0.43417						
	0.034						
	C						

IL8	-0.41465		
	0.0439		
	VDVAS I		
IL2	-0.42064		
	0.0407		
	VDVAS I		
IL5	-0.41159		
	0.0457		
	HAD A		
IL10	0.40744		
	0.0481		
	A	**VDVAS I**	**HAD D**
IL13	-0.45475	-0.44285	-0.43215
	0.0256	0.0302	0.035

Table 3.2.16 Summary of the Cytokine Correlations

Assessment Parameter	Negative correlation	Positive correlation
Spielberger – State anxiety	TNF-α	
HAD – Anxiety		IFN-γ, IL-10
HAD – Depression	TNF-α, IL-13	
EPQ R– Psychoticism	IL-6, TNF-α	
EPQ R– Addiction	IL-2, IL-13	
EPQ R –Criminality	IL-8	
VSI		IFN-γ
VDVAS-I – Intensity	IL-2, IL-5, IL-13	

Figure 3.2.15 Selected Whole Group Correlations



CHAPTER FOUR:

DISCUSSION

PSYCHOLOGY

Introduction

Interest in the psychosocial aspect of IBS is fuelled by the high prevalence of functional disease, the ambiguity surrounding the pathogenesis of symptoms and the lack of compelling treatment options. Symptom generation and propagation is likely to be influenced by many factors. Indeed the biopsychosocial model of disease proposed by Engel informs us that the phenotype of disease is a product of the synthesis of three domains affecting Self- the biological, the psychological and the social.[256]

The Study Dataset

Data produced from this study has demonstrated considerable psychological comorbidity in the IBS group. Most of the psychological parameters that the protocol employed produced significant differentiation between the study and control groups. This study was comprehensive in its breadth of psychological analysis. Most studies use 1 or 2 different psychometric devices. In contrast, our study protocol scrutinised symptom specific anxiety (Visceral Sensitivity Index), generalised anxiety (Hospital Anxiety and Depression Scale), contextual anxiety (Spielberger State Trait Anxiety Index), depression (Hospital Anxiety and Depression Scale; Spielberger State Trait Anxiety Index), somatisation (Patient Health Questionnaire-15), and hypochondriasis (Whiteley Index).

The study data showed that IBS patients were more introverted (Figure 3.1.3); neurotic (Figure 3.1.4); displayed a tendency to dissimulate (Figure 3.1.5); demonstrated more GI symptom-specific anxiety (Figure 3.1.8), generalised anxiety (Figure 3.1.11, Figure 3.1.14) and situational anxiety (Figure 3.1.); had a greater tendency towards depression (Figure 3.1.12); were scored more highly for hypochondriasis (Figure 3.1.10) and somatisation (Figure 3.1.9). There was a significant impairment in their day-to-day functioning (Figure 3.2.3). This data seems to be consistent with the literature on psychological co-morbidity in IBS.

Psychological Co-Morbidity in IBS

Psychological symptoms and coexisting psychiatric disease are common in IBS.[257] The prevalence of depressive or anxiety disorders in FGIDs is remarkably high (estimated to being between 42% and 61%.[258-263]). This relationship is even more pronounced in IBS patients seeking medical services.

Panic disorder, generalized anxiety disorder, major depression, social phobia and post-traumatic stress disorder were the most common anxiety-related psychiatric comorbidities associated with IBS patients who seek treatment.[264] Community-based studies dismiss the suggestion that treatment-seeking is a function of psychiatric disease- IBS anxiety-related psychiatric comorbidity in the non-treatment seekers appears to have similar prevalence rates compared to IBS treatment seekers.[265]

However, it should also be noted that psychiatric disease on its own is prevalent in community populations. Standard psychiatric interviews (using the Present State Examination) of new referrals presenting to a general medical outpatients revealed a 15% prevalence of psychiatric disorders in those with medically-explainable symptoms and between 38% and 45% prevalence in those patients with either poorly or unexplainable symptoms.[266] A Belgium study randomly selected 2316 patients in a primary care setting, identifying threshold or sub-threshold psychiatric disease in 42.5%, the most common conditions being major depression, anxiety and somatoform disorders.[267]

Life Stressors

Life stressors are reported more frequently in IBS patients.[268] Exacerbations of stress can be positively linked to increased symptomatology, health-seeking behaviour and illness-related absenteeism.[268] Recurrent or chronic stress produces a damaging sequence of physiological sequelae arising from heightened and persistent neuroendocrine arousal. Sterling and Eyer coined the phase *allostatic load* in 1988 to describe this.[269]

Allostasis

Allostasis is an adaptive mechanism designed to preserve physiological homeostasis in response to stress or threat. Therefore in the short-term its manipulation of various body systems (such as the immune system, the nervous system or the HPA axis) confers an advantage to the individual. Yet the inability to turn off this system promotes abnormal illness behaviour and disease. The concept of allostasis was refined by further work by McEwan and colleagues.[270,271] Clinical symptoms suggesting allostatic load include fatigueability, irritability and various somatic and visceral pain symptoms.

Early life events

Early life events may contribute to the development of disordered stress management. Such events may include emotional neglect, unstable relationships between parent and child or abuse in any of its forms. At an early age, this can lead to maladaptive stress responses leading to life-long psychosocial and medical dysfunction.[272,273] Dube et al. demonstrated that adverse childhood trauma increases the lifetime risk of attempted suicide between 2 and 5 times, and that it was also associated with the development of alcoholism, depression, illicit drug use and other high risk behaviour.[274] Other adult-onset medical conditions associated with early life events include COPD, autoimmune disorders, asthma, mood disorders, prescription drug use and obesity.[275-277]

Early life events and abuse have been documented with increased frequency in patients with IBS and functional disease.[67-70,75,278] Even if one disregards the ultimate diagnosis, patients reporting to GI clinics with functional symptoms experience greater pain severity, psychological distress, daily dysfunction and days off sick if an abuse history is present. From a clinician's point of view it is important to make a rapid diagnosis (for example, of a child presenting with recurrent abdominal pain) to allow the timely intervention of cognitive therapy (and parent re-education) to prevent life-long maladaptive illness behaviour.[279] Creed et al. offered a measure of consolation to this subject- IBS patients with a history of self-reported sexual abuse were more likely to respond to psychological therapy.[149]

Chronic Stress and Symptom Severity

Bennet et al. studied the relationship between chronic stress and the severity of IBS symptoms.[280] Life stress and symptom intensity measures were recorded over a 16 month period. They concluded that chronic highly threatening stressors influenced symptom severity. Those IBS patients faced with just one of said stressors did not display any clinical improvement during the testing period whereas *all* of those IBS patients without stress had a 50% improvement in symptoms. The relationship between stress and symptom generation was so strong that for the majority of IBS patients one could predict that if a critical stress threshold was breached, symptomatic improvement would be unlikely.

Previous research has shown that psychological factors are responsible for the development of functional disease. Gwee et al. demonstrated that life events and scores for hypochondriasis were

independent predictors of the development of PI-IBS twelve months *before* the primary gastroenteritic insult.[281]

The relationship between self-reported stress, psychological distress and GI symptoms has been repeatedly assessed by Heitkemper's group.[282,283] They documented positive correlations between mean daily stress and GI symptom distress, and an association between stress, anxiety and depression. Further work by the same group concluded that stress is a predictor of GI-related symptom severity, and that day-to-day fluctuations in the physiological response to stress are a component of symptom generation.[284]

Even in a population of healthy volunteers, studies have shown a positive correlation between symptoms consistent with functional GI disorders and the admission of perceived chronic stress, and the presence of dispositional stress reactivity and maladaptive coping strategies.[285]

Conversely the appropriate management of stress, and the introduction of new coping mechanisms to deal with chronic stress produce an improvement in the level of GI symptoms.[286-288]

Hypothalamic-Pituitary-Adrenal Axis

From a neuroendocrine perspective, the dysregulation of the HPA axis is implicated in the generation and propagation of IBS symptoms.[289-292] As an example, Dinan et al. demonstrated elevated basal cortisol levels and an exaggerated response to CRH injection in IBS patients compared to controls.[290]

Of the many HPA axis studies that have been performed in IBS patients, there remains some disagreement about basal cortisol levels between IBS patients and controls, and the stimulated stress response.[293] These differences may be attributable to study design, methodological differences or patient selection. However, the consensus appears to be that HPA axis dysregulation is a feature of IBS, and that most investigators report higher baseline and hormone-stimulated cortisol levels but variable HPA axis responses to physical or emotional stressors.[293]

Chang's group questioned the relationship between the HPA axis and early adverse life events.[294] They divided a group of IBS patients into those that had childhood maltreatment, and those that did not. They subjected both groups (plus a control arm) to the visceral trauma of sigmoidoscopy whilst serially measuring salivary cortisol levels. Their analysis concluded that HPA axis overactivity was more closely linked to the adverse childhood exposure than to IBS. In addition, the prompt return of

cortisol levels to baseline was associated with improved quality of life and less symptoms severity in the IBS group.

Maternal Deprivation Model

The maternal deprivation model is employed in animal studies to stimulate early life stress.[295] Although other models have been used (such as prenatal stress or early post-natal handling paradigms), the separation of a pup from the mother is the most widely used model to mimic early life stress and stimulate stress-related adult-onset disease. Maternal separation of murine neonates predisposes them to stress-induced colonic epithelial dysfunction (with increased intestinal permeability) and visceral hypersensitivity mediated by acetylcholine and CRF.[191,296,297]

An explanation of the link between early life conditioning and the subsequent response to stress (and the reactivity of the HPA axis) can be inferred from animal studies. Liu et al. used a murine model to show that the offspring of maternal rats who exhibited more licking and grooming of her puppies demonstrated reduced ACTH and cortisol responses to acute stress, and decreased levels of hypothalamic CRH transcription. Cross-fostering studies conclude that it is the nurturing of the mother rat (regardless of whether she has given birth to the baby rat) that produces this protective effect on HPA axis responses.[298] This introduces the concept of epigenetics- this is the idea that changes to phenotype or gene expression are not necessarily related to changes in DNA sequencing.[299] Changes in epigenetic programming are realised by changes in chromatin structure and methylation of DNA sequences, resulting in phenotypic plasticity.

In the context of the maternal separation studies, the suckling behaviour of the mother rat appears to program HPA axis reactivity in the newborn.[300-302] Furthermore, the unpredictability of early life trauma is paramount to the development of visceral hypersensitivity in later life.

Startle Responses

Other evidence linking the emotional state and centrally-driven over-arousability can be found in research using startle responses.[303] In this paradigm, the startle response is used as a non-invasive measure of the central defence response to threat. Naliboff et al. subjected IBS patients and controls to either abdominal or non-abdominal (bicipital) aversive stimuli during cued periods of safety (no stimulation given), imminent threat (possible stimulation), anticipated threat (a rest period before the next phase of imminent threat) and contextual threat (patients were informed that the

connections would be used to administer threat but that no stimulations would be given during this phase) whilst at the same time monitoring the acoustic startle responses.[304]

The data revealed that IBS patients mounted a larger startle response during anticipation and imminent threat (after an initial stimulation had been given). Psychometric analysis demonstrated that neuroticism (as defined by Eysenck Neuroticism scale) was associated with larger startle responses during periods of safety and anticipatory threat than during imminent threat, and that anxiety was negatively correlated with startle responses during imminent threat. The importance of this work is that even though peripheral visceral hypersensitivity is an important pathological mechanism in IBS, central structures, circuits involved in the emotional response to aversive or non-aversive stimuli and one's psychological constitution are intrinsically linked to the perception of pain.

The Limbic System

The central pathway that is responsible for integrating sensory perception and emotion is the limbic system, and in particular the amygdala.[305] This structure determines the emotional context of afferent sensory nerve transmission, and serves to acquire and maintain memories pertaining to said sensory stimulation. As a result the amygdala is a key pathway in forming the fight or flight response, and for the creation of anxiety and fear. Its role in the conditioned response is undisputed.[306,307] The advent of more advanced imaging techniques such as functional MRI confirms its importance in the modulation of pain.[308]

Manipulation of the limbic system can be performed using simple laboratory paradigms.[309] Healthy volunteers were asked to review various unpalatable and disturbing images to induce a state of negative affect. Subsequent testing demonstrated enhanced startle responses, and diminished inhibitory pain pathways.[310]

The Role of the Amygdala

A recent study by Choi et al. devised a system of dynamic fear arousal in which rats were subjected to the emotional trauma of a having a predatory robot programmed to lunge at them whilst they were foraging for food.[311] The initial behaviour was that of fleeing to the nest or freezing. Chemical disinhibition of the amygdala (using an intra-amygdalar infusion of a $GABA_A$ receptor antagonist) prompted heightened fear responses in the rats. Conversely the chemical inactivation of the amygdala (using a $GABA_A$ receptor agonist infusion) caused the rats to abandon any escape response when confronted by the lunging robot.

This study gives a valuable insight into the bidirectional role of the amygdala in risk-taking behaviours. Further support for this can be seen by studying patients with bilateral lesions of the amygdala- De Martino et al. subjected two such individuals to various experimental economic tasks, asking them to gamble, thereby exposing them to the risks of losses in the pursuit of gains.[312] Compared to controls, they demonstrated a remarkable reduction in loss aversion, the concept of forgoing the opportunity of substantial reward due to the fear of realising a loss.

Dysregulation of the Amygdala

Dysregulation of the amygdala and its connection with the ventromedial prefrontal cortex, a central structure involved in sympathetic arousal, is associated with altered regulation of affect. Anxiety states such as Post-traumatic Stress Disorder have been shown to have enhanced reactivity of the amygdala to affect-inducing stimuli with concomitant suppression of the ventromedial prefrontal cortex (indicating increased physiological arousal).[313,314] This has led to the hypothesis that altered activity in the amygdala and associated pre-frontal structures is the pathological foundation for the development of anxiety states.

In the context of IBS, Naliboff et al. demonstrated enhanced responses in the amygdala and the dorsal anterior cingulate cortex in response to anticipated and delivered rectal balloon distension.[315] The same group published a companion study which documented that repeated visceral stimulation with the barostat (six sessions over a 12 month follow-up) produced habituation of the visceral perception of rectal distension-[316] PET scanning elucidated that this correlated with decreased activity of the amygdala, the dorsal anterior cingulate cortex and the dorsal brainstem.

Emotional Over-arousal

Petrovic et al. suggested that when the body is faced with an imminent painful threat, suppression of the amygdala and its connections is part of a cognitive coping strategy.[317] The failure to down-regulate the activity of the amygdala during anticipated pain, and to maintain a state of emotional over-arousal may be a hallmark of IBS and other chronic pain states.[318] Mayer et al. characterised this as “ineffective cortico-limbic-pontine inhibition of visceral afferent input within the homeostatic afferent processing network”.[319] How this then translates to IBS GI symptomatology can be inferred from animal studies which show that a conditioned fear environment produces increased colonic motility in rats.[320]

Somatisation

Many studies report an association between functional disease and somatisation.[321-324] The increasing number of functional diagnoses made in any one individual should give rise to consideration for the presence of somatoform disorders. The criteria for the diagnosis of Somatisation Disorder (SD) has features which complement the diagnosis of IBS. Again mirroring IBS, SD uses subjective patient reports to arrive at a diagnosis. Using DSM IV classification,[325] Somatisation Disorder is defined as a persistence of physical symptoms in the absence of a medical explanation. There are five features: a history of somatic symptoms prior to the age of 30, two GI-related symptoms e.g. diarrhoea, pain in at least 4 different parts of the body, one pseudoneurological symptom e.g. fainting and one symptom of sexual dysfunction e.g. erectile dysfunction.

Somatisation and IBS

Clearly the overlap between SD and IBS is obvious. A study by North et al. was designed to tease apart the strands of functional disease from primary somatoform disorder and other psychiatric illness in a female IBS population attending outpatients[323] SD was confidently diagnosed in 25% of patients with another 5% being labelled as having a high probability of having the disorder. This has significant implications in the construction of clinical trials, and perhaps SD DSM IV-positivity should be screened in future research models.

Somatoform disorders were traditionally assumed to be an external manifestation of psychological disharmony.[326,327] However, other theories such as visceral and somatic hypersensitivity or a cascade of catastrophic cognitions and overemphasised fears (i.e. thinking that a transient episode of chest pain is synonymous with having a heart attack) may act as mechanistic drivers of disease. It is important to note that the strict criteria for diagnosis does not make reference to psychological comorbidity.

Another interesting observation is that even though SD is not defined by psychological parameters, patients with SD may describe psychiatric symptoms more frequently than patients with known non-somatoform psychiatric disease. Lenze et al. documented a wide variety of psychotic, manic, depressive and anxiety-related symptoms in a group of SD patients that was not attributable to other psychiatric comorbidity.[328] Indeed patients with SD were found to have increased frequency of current and lifetime psychiatric symptoms than those with Cluster B personality disorders

(comprising the Histrionic, Narcissistic, Antisocial and Borderline Personality Disorders). Again the caveat to this study was that SD should be an important differential diagnosis for someone presenting with wide-ranging psychiatric symptomatology.

Irritable Bowel Syndrome patients with the comorbidity of a somatoform disorder tend to have more marked abnormal illness behaviours e.g. increased utilisation of healthcare resources, days off work, changes to medications, and display more psychiatric symptoms.[323]

Diagnostic uncertainty arises when one has to make a diagnosis of IBS in the presence of somatoform symptomatology, especially if the patient does not meet the precise criteria for Somatisation Disorder or one of the other somatoform disorders (undifferentiated somatoform disorder or somatoform disorder not otherwise specified). Or if a patient with known psychiatric disease presents with medically ambiguous symptoms. Furthermore, the magnitude of somatoform symptoms may not be discernible from a single doctor/patient consultation, and the diagnosis or diagnoses may involve a considerable amount of time and effort on the part of the clinician.

Conclusion

Our study endorses the well-documented associations of psychological comorbidity in Irritable Bowel Syndrome. Knowledge of these associations, and the differentiation of a primary psychogenic process from a functional gastrointestinal process with multifaceted psychological dimensions are crucial to the management of the patient. The use of psychometric questionnaires should be considered a vital core of any future clinical trials involving IBS. Finally, the observation of more centrally driven psychologically-mediated symptoms in refractory IBS should alert the physician that psychological manipulation, for example with hypnotherapy or cognitive behavioural therapy, may be a worthwhile consideration.

NEUROPHYSIOLOGY

The Study Dataset

The results from our data have shown that rectal electrical stimulation produces a robust cortical response that is stable and reproducible over time (Tables 3.1.22b to d). This is the first study to demonstrate such findings in the IBS population. As shown in Figures 3.1.19 to 3.1.22, the evoked potentials were compared at many time points- during each cycle of testing, during separate cycles (intra-day), and during different days of testing (inter-day). There was no statistical difference between data collected from either patient or control subject during intra-day comparisons. In other words, when someone is being tested on a particular day the first cycle of evoked potentials will be comparable to the last cycle of evoked potentials. This has important implications in that one could dramatically shorten the test protocol from 4 cycles to 2 cycles (or even less).

Anorectal Sensitivity

Testing both IBS patients and controls over 3 separate days allowed for serial assessment of neurophysiological function. Examination of the sensory threshold data showed that the IBS group appeared to be less sensitive on Day 2 compared to controls. However, the data from Days 1 and Days 3 is similar, and my assumption is that the sensory threshold data is an anomaly produced from a few outlying results.

Pain threshold testing on the first two days did not show any statistical difference between controls and cases. This echoes previous studies that show that in a pre-excited state it can be very difficult to separate IBS patients and controls.[329] A difference in pain thresholds was noted on Day 3. This showed that whilst the IBS group maintained a fixed pain threshold, the control group became more disinhibited, allowing a greater electrical stimulus to be applied. This is the concept of habituation, in which one becomes conditioned to a repetitive aversive stimulus. Once more this raises speculation that IBS patients may lack the ability to disinhibit themselves, or conversely, to activate descending inhibitory pathways leading to symptom persistence and reinforcement. Perhaps the manipulation of descending inhibitory pathways and the stimulation of the endogenous opioid system is a potential therapeutic target.

CEP Morphology

The morphology of the evoked potentials (Figures 1.3.4, 3.1.19 to 3.1.22), as defined by the principle components of the evoked response, though different in amplitude and latency, maintained a similar

morphology in all subjects tested. In some individuals, the evoked potentials were almost indistinguishable between separate days of testing. Of course, different people produced different CEP traces. This arises from many factors- for instance, the variation in the amount of electricity used, changes in the integrity of the visceral sensory neural pathways, the preparation of the bowel, and the degree of anxiety experienced in the run up to the procedure.

CEP Response to Increased Stimulus

The evoked potentials were exquisitely sensitive to the amount of stimulus applied. As the stimulus intensity increased, the P1 amplitude increased and the P1 latency decreased. This is a commentary on the increasing recruitment of sensory afferents and the more dominant cortical representation of the stimulus as it increases. It also reflects the fact that different nerve fibres have different myelin configurations.

Those afferents that are not myelinated, such as C fibres, have a higher activation threshold than their myelinated counterparts, e.g. Aδ fibres. As a result there is preferential activation of Aδ fibres at low stimulus intensities with increasing C fibre depolarization at the higher stimulus intensities.[330] Further evidence of the preferential firing of Aδ fibres is supplied by Bromm et al. This group demonstrated that C fibre afferent transmission was much slower than Aδ fibre transmission, and accounted for a late and ultra-late CEP response (>1000ms).[331-333]

P1

Hobson et al.'s comparison of the cortical representation of MEG and CEP data following painful (visceral) oesophageal stimulation predicts that the P1 wave seen in our own data represents the firing of the primary (S1) and secondary (S2) somatosensory cortex and the posterior insula.[334] Their data showed P1 activation at around 85ms, and increased activity of the anterior insula and the cingulate cortex at around 105ms. Our data has a P1 latency of 73.5ms in controls and 96.35ms in IBS patients on the first day of testing, suggesting that there is a consistency between the two studies.

The Hobson paper also reflected on the fact that neural activity was maintained for a longer period of time on the CEP tracings than the MEG. This may explain why a discrete peak following P1 (and before P2) was not seen- this would be consistent with the firing of the anterior insula and ACC.

Early and Late CEP Complexes

The rectal evoked potential has been described in terms of its morphology and the number of peak deflections from the baseline, but also in terms of latency. The early CEP complex occurs within 250ms, and represents the initial cortical activity following the applied stimulus. This is said to be the exogenous response of the stimulus-induced neural activity.

Subsequent CEP activity beyond 250ms reflects the endogenous processing of the stimulus- this is the late CEP complex. It represents the analysis of the stimulus amongst higher cortical domains of pain perception, perhaps invoking the emotional, behavioural or attentional centres.

P2 and P3

Peak 2 and Peak 3 produce a large scale explosion of neural activity following the stimulus. In the Hobson paper, this activity was seen across all the topographical areas of testing, including the somatosensory cortex (S1, S2), and the anterior and posterior insula cortex. [334] Given that CEPs can only record the volume of neural activity (i.e. amplitude) rather than any anatomical differentiation, the large P3 peaks represent combined neural activity of all these areas. In both controls and IBS patients the P3 latency occurred with 250ms. The decay of the P3 potential marks the transition of the early CEP complex into the late CEP complex.

Late CEP Complex

The late CEP complex is interesting because research has shown that cortical evoked potentials can be seen even in the anticipation of a visceral stimulus. Hollerbach et al. demonstrated that anticipatory evoked potentials with peak latencies of around 280ms were produced in control subjects experiencing oesophageal visceral stimulation with intermittently (and randomly) non-firing pauses.[254] The authors concluded that this late phase of cortical activity may be a surrogate marker for the cognitive processing attached to visceral perception. Our data suggests that this neurophysiological profile is consistent with a hypervigilant phenotype. Clues to this phenotype during testing would be an IBS patient with high STAI scores, low stimulus intensity, protracted latencies but robust late CEP complex tracings.

Combining Pain Threshold with Peak Latency

Hobson et al. used CEPs to differentiate in neurophysiological terms a group of patients with non-cardiac chest pain.[209] Despite the fact that these patients shared the same clinical presentation and

had similarly negative workups for oesophageal manometry and pH testing, the use of CEPs was instrumental in delineating three discrete sub-populations.

The first group had reduced pain thresholds to electrical oesophageal stimulation and normal/reduced P1 latencies. The second group had reduced pain thresholds with increased P1 latencies. The final group had normal pain thresholds with normal (or increased) P1 latencies. They concluded that the first group were demonstrating a phenotype with enhanced oesophageal afferent sensitivity. The second group of patients reported pain or discomfort with delayed P1 latencies, indicating an absence of afferent hypersensitivity. Symptom generation in the latter group was most likely secondary to cortical amplification of a normal afferent stream.

Sub-Group Analysis of the IBS Dataset

The pairing of anorectal sensitivity with CEP latency can be applied to our own dataset. This allows sub-differentiation of the IBS group. It should be noted that this differentiation is not possible without the use of a subjective record of patient pain perception- CEPs viewed in isolation could do nothing more that demonstrate whether there is normal or delayed afferent transmission for a given stimulus. The additional of an individual's experience of the applied stimulus informs one how to interpret the CEP traces.

Proposing a Reference Range for Pain Threshold and CEP Latency

In order to identify in neurophysiological terms what is abnormal, one has to define what is normal. P1 latency was taken as the reference latency. Conceptually it seemed more logical that the first representation of the cortical response should be used as the defining variable. However, using P2 latency would have been equally appropriate.

The control 3 day mean P1 latency was 73.4ms with a standard deviation of 16.1ms. Two standard deviations above this is 106ms. Values above 106ms were considered to be attenuated. The cut off for pain threshold was defined as being less than 50mA since all healthy controls had levels above this value (range 50 to 90mA).

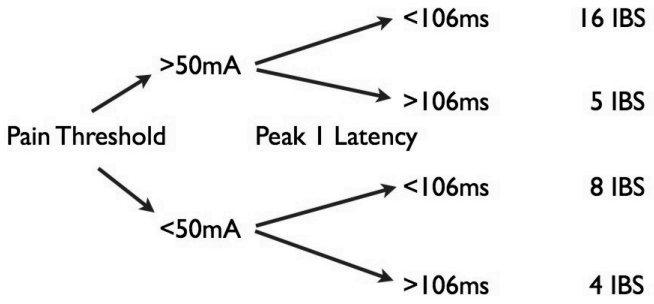
Determining an appropriate value for the pain threshold was more difficult than for the P1 latency. 1 SD below the control pain threshold was 48.19mA, and 2 SDs below the control pain threshold was 26.33mA. If we had used the same system- projecting 2 SDs below the control mean pain threshold- most if not all of the IBS patients would have been grouped amongst the control data. However, a

decision to define the pain threshold as being less than 50mA, whilst not as statistically robust, still identified a small sub-group of IBS patients that had thresholds below that of their control counterparts.

Identifying the IBS Subgroups

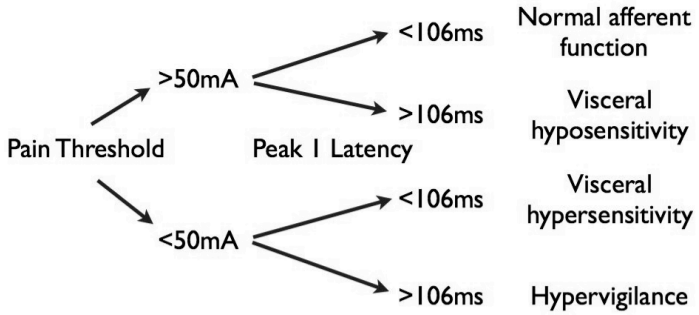
Twelve IBS patients had a mean three day PT ≤50mA: four patients had P1 latency >106ms, and eight patients had a P1 latency ≤106ms. Twenty-one patients had a mean three day PT ≤50mA: five patients had had P1 latency >106ms, and sixteen patients had a P1 latency ≤106ms. This data is summarised in Figure 4.1.

Figure 4.1 Patient Stratification using Pain Threshold and Peak 1 Latency



Extrapolation of this data suggests that of the IBS patients that were tested, 16 of the 33 IBS patients had normal visceral afferent function (normal P1 latency and normal sensory thresholds), 4 patients were hypervigilant (decreased sensory thresholds with delayed P1 latency), 5 patients were hyposensitive (raised sensory thresholds with delayed P1 latency) and 8 patients demonstrated visceral afferent hypersensitivity (decreased pain thresholds with decreased P1 latencies). This data is summarised in Figure 4.2.

Figure 4.2 Identifying the IBS Subgroups using Pain Threshold and Peak 1 Latency



Neuropsychophysiological Patterns

While it would be difficult to present all the CEP traces for 21 Controls and 34 IBS patients spanning 3 days, three CEP patterns emerged. These patterns must be viewed in the context of the amount of electrical stimulus used, and the prevailing anxiety state.

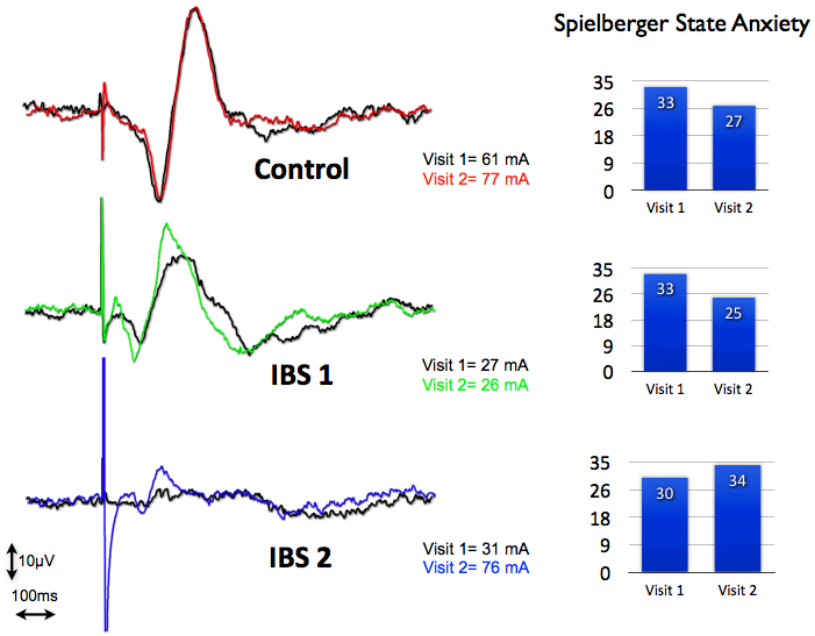
The first pattern is what would be considered the normal pattern. This can be seen in Figure 4.3., labelled Control. The Control demonstrates a good cortical response over 2 separate days using stimulus intensities of 61 and 77mA respectively. The anxiety state is defined by the Spielberger State Anxiety Index. In this subject, anxiety drops from Day 1 to Day 2 as might be expected of someone becoming more relaxed about the testing process.

The second subject, labelled IBS 1, produces high amplitude, short latency evoked potentials in the context of dropping anxiety levels and tiny amounts of electrical stimulus (27 and 26 milliamps on separate days). This is very much the pattern that we expected to see in primary rectal afferent hypersensitivity. It reflects an upregulation of visceral afferent transmission, one of the putative driving mechanisms of IBS symptomatology.

The third subject, termed IBS 2, produces ill-defined CEP traces on the first day. Only 31mA of electricity was applied. By Day 2, the subject manages to tolerate a much higher electrical stimulus even in the context of increasing anxiety levels. The distinction here is that the patient can tolerate high electrical stimulations (someone with visceral hypersensitivity would not tolerate such a jump in electricity), but was not able to do so on Day 1. This is independent of any dysfunction in the afferent stream- when the increased current is applied on Day 2 the CEP trace starts to emerge from

the ether of background cortical electricity. This kind of IBS patient personifies the chronically aroused, hypervigilant phenotype.

Figure 4.3 Cortical Evoked Potentials from a Control subject and two IBS patients on Day 1 and 2



Summary of CEP data

In summary the study protocol has managed to define 4 distinct subgroups based on objective neurophysiological and anorectal parameters. The fact that only 8 of the 33 (approximately 25%) have been found to have rectal afferent hypersensitivity may appear somewhat less than the prevalence described in other IBS literature. Some studies calculate the prevalence of hypersensitivity to balloon distension in IBS patients at around 60%.[335,336]. The presence of rectal afferent hyposensitivity at around 15% is consistent with established data. Observations from Professor Whorwell's group (and other research groups) demonstrated that a small minority of constipation-predominant IBS patients (between 6 and 19%) had rectal hyposensitivity to balloon distension.[138,337]

This does not discredit either the technology of CEPs (and sensory threshold testing) to identify patients with rectal afferent hypersensitivity, or the concept that visceral hypersensitivity is a

hallmark of IBS. It can be explained by the fact that IBS reflects a heterogeneous population, that previous research using the barostat introduces confounding factors at every step of the way from the peripheral receptor to the cortex (thereby embellishing the prevalence of visceral hypersensitivity), and because visceral hypersensitivity is the final expression of a complicated pathway that involves the modulation of the afferent sensory stream by spinal, sub-cortical and cortical processes.

In order to scrutinise the role of visceral hypersensitivity in IBS, it is important to observe its conceptual development, and to identify each step of the afferent pathway towards the S1/S2 cortex.

Dismissing Old Theories

Many studies now conclude that visceral hypersensitivity is an important pathological mechanism in Irritable Bowel Syndrome.[338] However, before the concept of visceral hypersensitivity became popular, other theories on the development of symptoms in IBS were prevalent. The most notable was the theory of colonic dysmotility leading to symptom generation. However research designed to investigate colonic dysmotility failed to correlate changes in colonic motility with the onset of pain.[339] As a result, the theory of visceral hypersensitivity started to become more conceptually relevant.

The Era of the Barostat

The demise of the theory of dysmotility introduced the era of the barostat in which the tolerance to rectosigmoid balloon distension was seen as a biomarker for colonic sensitivity.[336] Many research groups documented increased colonic sensitivity in IBS groups compared to controls using the barostat, or have shown that repetitive stimulation precipitates a sensitised state.[329]

Some investigators suggested that the aberrant referral of pain to non-sacral sites during barostat testing was a characteristic of dysregulated sensory function. This has been termed aberrant viscerosomatic referral.[340] Other groups have demonstrated visceral hypersensitivity in other parts of the gastrointestinal tract in the absence of rectal hypersensitivity. This may reflect regional and/or temporal changes in visceral hypersensitivity.

Taken in isolation, hypersensitivity to rectal balloon distension can identify the majority of IBS patients being studied.[336] The addition of two other variables, namely the pattern of viscerosomatic

referral and the intensity of perceived sensations improves the sensitivity of barostat testing. 94% of IBS patients were identified in Mertz et al.'s IBS cohort using this methodology.[336] However, regardless of the barostat test paradigm, it is impossible to remove the psychological and emotional processing associated with having an inflatable balloon inserted in the rectum.

Subjective Sensory Perception

Whitehead et al. scrutinised the phenomenon of subjective sensory perception during balloon distension.[341] They noted that IBS patients reported more pain even during sham balloon distensions; in addition, laboratory measures taken to reduce psychological factors during balloon distension caused a shift of the IBS hypersensitive state to that of normal visceral sensitivity. Furthermore, psychological remodelling, for instance via hypnotherapy, has been shown to reduce rectal sensitivity, clearly demonstrating the involvement of central processes in the functioning of ostensibly peripheral mechanisms in pain perception.[138]

Visceral Afferent Sensory Transmission

It is quite clear that it is difficult to separate peripheral visceral signalling from the emotional or environmental context of the stimulus. Visceral afferents travel towards the dorsal horn in association with sympathetic fibres via the splanchnic nerves passing through the mesenteric ganglia and the prevertebral ganglia. The exceptions to this are the visceral afferents supplying the anorectum that travel via the pudendal nerves, and those of the proximal gut (from the oesophagus to the jejunum) that ascend via the vagus nerve, terminating in the Nucleus Tractus Solitarius. Any exaggeration or inhibition of any part of this sequence may provoke a hypersensitive (or hyposensitive) state. In effect, changes to peripheral nerve transmission, central sensory processing or descending efferent nerve transmission may alter the perception of pain.

Visceral Noiciceptors

A widespread yet low density network of noiciceptors populates the mucosa of the gut. Visceral afferents do not populate the gastrointestinal tract in isolation- they also innervate the mesentery, heart and other viscera, and serve as a communication pathway between the central and enteric nervous systems. Furthermore the proximity of afferent fibres to inflammatory cells e.g. mast cells or enterochromaffin cells may suggest the link between the visceral pain pathways and the immune system.[194,342]

Visceral Afferent Receptors

When a peripheral stimulus is applied, many different types of receptor may be instructed to convey the signal. Visceral afferent receptors are divided into 3 classes- intensity-encoding mechanoreceptors, high threshold mechanoreceptors and mechanically-insensitive mechanoreceptors (the so-called "silent fibres").[343,344] The first group of mechanoreceptors have a low threshold for firing, and operate under normal physiological conditions. They encode both non-noxious (physiological) and noxious stimulus intensities. The second group have a high threshold for firing, and only encode noxious stimuli i.e. at intensities approximating to the pain threshold. The mechanically-insensitive receptors have very poor response to mechanical stimulus but are sensitive to chemical, metabolic, inflammatory or thermal stimuli.[345-348]

In the context of the barostat, balloon distension produces a barrage of sensory information that involves multiple visceral receptors, ranging from those that are mechanically-sensitive to those that are not. In terms of being a discrete assessment of visceral hypersensitivity, there are many disadvantages to its use.

In contrast our study protocol employed a short-lived electrical stimulus that was programmed so that the stimulus was delivered at slightly different successive time points, thereby reducing anticipatory bias.

Disruption of the Peripheral Signal

Disruption or manipulation of peripheral sensory mechanisms highlight their importance in the perception of pain. For instance, the rectal mucosa can be artificially sensitised by agents such as glycerol[349] or desensitised by lidocaine.[350] In a more obvious clinical setting, bacterial sensitisation of the bowel produces an IBS symptom complex following gastroenteritis. It is also important to point out that IBS is characterised by visceral hypersensitivity but not somatic hypersensitivity. Accarino et al. went so far as to show that IBS patients had increased somatic pain tolerances compared to controls.[351] Fukudo et al. demonstrated exaggerated colonic motility in IBS patients using a barostat distension paradigm that fell below the sensory threshold, suggesting an abnormal pre-cortical reflex.[352]

Visceral Pain

The perception of visceral pain is ambiguous, ill-defined and non-specific. In the clinical context, patients with IBS may report recurrent abdominal pain of variable character, intensity and radiation.

These symptoms may be difficult to distinguish from other clinical conditions. This is due to the anatomy of the visceral neural pathways.

Visceral Receptor Convergence

1st order neurons have diffuse connections over multiple segments of the spine.[353] Visceral noiciceptors converge with spinothalamic projection neurones in lamina I, II and V of the dorsal horn.[354] The spinothalamic neurones receive concurrent innervation from somatic afferents from both deep and superficial somatic structures in the same dermatome. This anatomical arrangement explains the phenomenon of referred somatic pain.[355] This occurs when noxious stimulation of visceral afferents co-stimulates viscero-somatic neurones at the level of the dorsal horn precipitating dermatomal pain. It may also explain why people who complain of symptoms from non-gastrointestinal viscera (e.g. dysmenorrhoea) demonstrate heightened sensitivities during colonic distension testing.

Other patterns of convergence occur at the dorsal horn between visceral afferents and autonomic (vagal) and enteric fibres leading to further blurring of the clinical picture- for example the onset of visceral discomfort may invite an autonomic response, such as nausea, sweating and vasomotor flushing.

Subliminal Processing of Visceral Signals

Most visceral afferent sensory flow does not reach the level of cognitive processing, i.e. the background level of visceral activity is filtered out before reaching the consciousness. This allows the individual to concentrate on more pressing cognitive demands. The exceptions to this are the sensations of fullness required to alert the body that the point of satiety has been reached or that it is time to void. However, during times of pain, biochemical stress or inflammation, there is an increased firing of the high-threshold receptors (and/or chemoreceptors) to signal distress.

Homeostatic Afferent Processing Network

Visceral signals ascend in the CNS where their information is processed by sub-cortical and cortical structures. Lamina I neurons in the dorsal horn project to those centres involved in visceral homeostasis. These structures include brainstem sites, parabrachial nucleus, periaqueductal grey, the caudal and rostral venterolateral medulla, hypothalamus and amygdala. Mayer et al. termed this the Homeostatic Afferent Processing Network.[319] Visceral information assimilated into this system is

processed initially by a hierarchical framework of modality-specific sub-cortical reflexes, both autonomic and somatic, designed to preserve homeostasis.[356]

In addition to sub-cortical processing, the Lamina 1 outflow is directed towards the cortex via the posteromedial aspect of the thalamus, designated the ventral caudal part of the medial dorsal nucleus (MDvc). This circuit activates the dorsal anterior cingulate cortex (dACC) which has a role in the cognitive affective appraisal of visceral (and somatic) sensation. It also serves to regulate autonomic function, emotional responses and behavioural motivations such as reward-anticipation.

Another discrete neural circuit projects visceral information to the Dorsal Posterior Insular Cortex (via the postero-lateral thalamus, termed the VMpo) which has responsibilities for temperature regulation, pain, hunger etc.[357]

Figure 4.4 Neuroanatomy of the Ascending Visceral Afferents

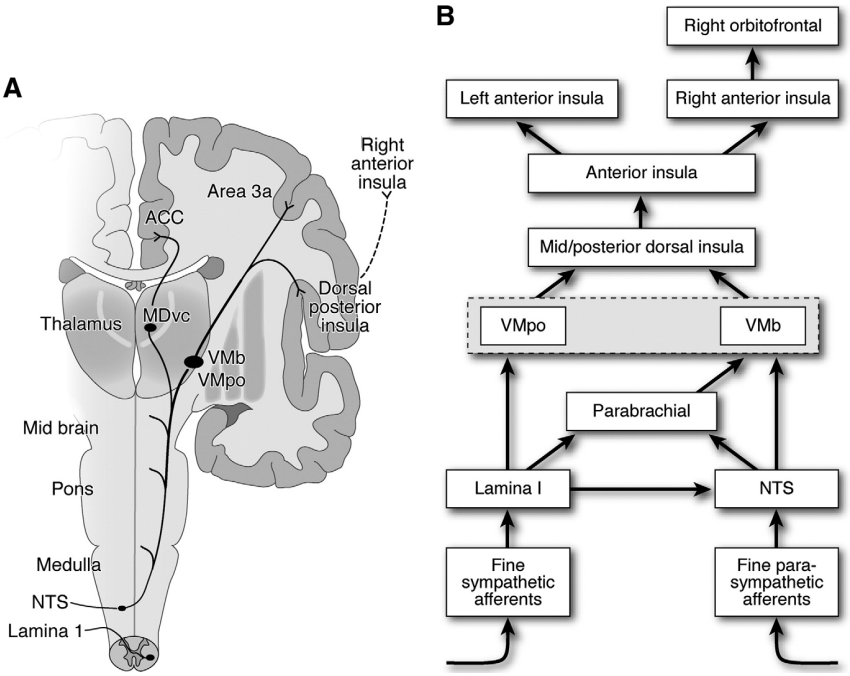


Figure A demonstrates the ascending projections from homeostatic afferents from the spine via Lamina 1, and from parasympathetic nerve fibres via the Nucleus Tractus Solitarius (NTS). This information convenes at the Parabrachial Nucleus before projecting to the venteromedial thalamic nuclei (VMpo and VMb) or the medial dorsal nucleus (MDvc). Subsequent projections activate the dorsal anterior cingulate cortex (dACC), dorsal posterior insula and area 3a of the cortex, part of the sensorimotor cortex. **Figure B** demonstrates ascending projections from homeostatic afferents towards the insula. Both figures adapted from Craig D, 2003.[358]

Sensory information passing through the dorsal posterior insula is relayed to the middle insula and (non-dominant) right anterior insula. The right anterior insula has intimate connections with the dorsal anterior cingulate cortex and the right orbito-frontal cortex, and mediates interoceptive awareness and the emotional response. The right anterior insula cortex is sensitive to homeostatic emotions (the awareness of the "physiological self") such as hunger, thirst, libido and temperature and affords them their subjective emotional context (e.g. happiness, anger, fear etc.). As a result, it is possible to define in neuroanatomical terms how a distant visceral signal can pass through layers of sub-cortical and cortical brain networks, culminating in a subjective, integrated emotional response.

Visceral Perception

The perception of visceral discomfort or pain is the translation of visceral sensory input by supraspinal structures into a subjective form that is influenced by memory, context, emotion, motivational factors and the level of cognitive processing. Dysregulation of any of these networks may cause altered perception of the visceral signal.

Other Research Modalities

The holy grail of visceral sensitivity testing in IBS is to mate an optimal method of stimulating the gastrointestinal tract with an optimal method of assessing the physiological response to the applied stimulus, preferably at multiple levels- the periphery, the spine and the brain. Of course this is unlikely to materialise. Many testing modalities have been put forward as being superior to their counterparts- these include functional MRI (fMRI), positron emission tomography (PET), single photon emission computerized tomography (SPECT), magnetoencephalography (MEG) and, of course, cortical evoked potentials (CEPs).

Functional Brain Imaging

The combination of functional brain imaging and visceral stimulation (regardless of modality) creates an anatomical representation of the regional areas of the brain responsible for the processing of visceral sensory information.[359] Functional MRI uses cerebral blood flow as a marker for neuronal activity i.e. activated areas of the brain stimulate increased blood flow. In contrast CEPs can show the magnitude of a cortical response, but cannot differentiate which parts of the cortex are being stimulated. On a rather simplistic level, the latency of the CEP (in the early phase of the CEP

complex) can highlight any delay in afferent transmission to the sensory cortex. The value of using either fMRI or MEG is that one can then see which specific areas of the cortex are being targeted.

Functional MRI and Subliminal Distension

Lawal et al. used functional MRI (fMRI) and subliminal distension pressures to test visceral processing in IBS patients.[360] The reason for using sub-threshold pressures was reduce the degree of stimulus-induced cognitive bias. Their data demonstrated increased activity in five cortical domains- the cingulate gyrus, the sensory/motor cortex, the parietal/occipital cortex, the pre-frontal cortex and the insular cortex- compared to controls. The mechanism for this is unclear- Azpiroz et al. suggested that it was either due to increased signalling from the peripheral afferent receptors, amplification of a normal peripheral signal in the spinal cord or due to central amplification of the signal.[361]

Pain-facilitating and Pain-inhibitory pathways

Mayer et al. sought to assess the contribution of central pathways to the development of visceral hypersensitivity by comparing IBS patients with quiescent colitics and controls.[362] Using the barostat and fMRI, they discovered that IBS patients showed enhanced activation of the limbic and paralimbic systems (comprising the amygdala, hypothalamus, dorsomedial pre-frontal cortex and ventral/rostral anterior cingulate cortex) compared to disease and non-disease control groups. Furthermore there was suppression of the periaqueductal grey in IBS patients, a descending pathway involved in pain inhibition. This study proposed that IBS patients had up-regulated pain-facilitatory pathways and diminished pain-inhibitory pathways. This finding was echoed in later work by the same research group. They discovered that IBS patients failed to activate pain-inhibitory pathways in the dorsal brainstem, namely the locus ceruleus and parabrachial nuclei, in response to an anticipated visceral stimulus.[318]

Whilst fMRI (and PET) has dramatically enhanced our knowledge of the neuroanatomical and neurometabolic representation of cortical pain processing, its use in IBS research is confined to the larger research houses. This is due to the capital expense of the equipment, and the associated costs of running the scanner. However, apart from the expense involved, fMRI is limited by the claustrophobic confines of the scanner and its sensitivity to artefact, anticipation and habituation.[316]

The principle failure of fMRI is its delayed temporal resolution (approximately 4 to 8 seconds). A peripheral stimulus may be applied, and the fMRI will flash up an area of increased tissue perfusion.

However, because cortical pain processing is an instantaneous and dynamic process, it cannot differentiate between stimulus-specific activity and stimulus-independent activity.[333]

Magnetoencephalography

Magnetoencephalography records minute magnetic depolarisations that occur in the cortex following electrical neuronal activity. This occurs in real-time.[363] In many ways it is the best method to investigate cortical processing because of its temporal and spatial resolution. However, there are few centres in the UK that offer it. Furthermore, there is good evidence to show that CEPs demonstrate cortical responses with temporal resolution that match that of MEGs.[334]

Cortical Evoked Potentials

Our study chose to use CEPs because we identified a clinical need in being able to differentiate IBS patients with pain-amplifying peripheral sensitisation from those with more centrally-driven processes. This has a clear cut utility in altering patient management- the clinician may consider to use psychological therapy as opposed to anti-spasmodics or analgesics if there is compelling evidence that the central over-interpretation of visceral information (as evidenced by a hypervigilant anxiety-driven profile) dominates the clinical picture. The reliance on the subjective reporting of pain and discomfort cannot be used alone since it is under constant threat from response bias, the tendency to over-report pain in patients with functional gastrointestinal disorders,[341,364] and the increased perception of visceral stimuli during stress in IBS patients.

Advantages of Cortical Evoked Potentials

Cortical evoked potentials have been thoroughly tested in the discipline of neuroscience. Their use as neurophysiological markers of visceral afferent nerve transmission and subsequent cortical processing has been recently described in a study on non-cardiac chest pain.[365] Evoked potentials have exquisite temporal resolution (recorded in milliseconds), the equipment is inexpensive and testing can be easily performed in a standard clinical setting. Reproducibility of the data can be seen instantaneously on a stimulus to stimulus basis. The learning curve to achieve competency in harvesting CEPs is short- this makes the training of technicians an expedient process. Furthermore, even in the presence of an electrical visceral stimulus, the testing paradigm is not perceived to be as onerous or as invasive as barostat testing.

Disdvantages of Cortical Evoked Potentials

Disadvantages to using CEPs are the time-consuming nature of the test protocol, and the importance of having at least 2 separate days of testing to allow for the initial novelty and associated apprehension of the procedure to subside (as evidenced by high anxiety scores for both controls and cases on the first day of testing).

Conclusion

In summary, our data shows that CEP testing in IBS produces reliable and reproducible data, and when twined with psychological evaluation and subjective responses to the applied stimulus, can give valuable insights into the putative mechanisms at play.

IMMUNOLOGY

Introduction

Cytokine analysis in IBS has come under increasing scrutiny in the field of IBS. Recent studies have demonstrated that differentiation can be made between IBS patients and control groups at an immune level.[182,186,187] It is postulated that IBS patients have a low grade inflammatory process that may contribute to the sensitisation of the GI tract, and contribute to the development and maintenance of the symptom complex.

The study by Gonsalkorale et al. acknowledged that the immune system was a delicate balance of pro-inflammatory and anti-inflammatory cytokine cascades. Their data suggested that in IBS this balance has become imbalanced, giving a more pro-inflammatory expression.[189] This aroused the prospect of being able to identify IBS patients from an immunohistochemical profile. The concept seemed to be attractive given that in other disease states such as inflammatory bowel disease or rheumatoid arthritis, the targeted use of cytokine antagonists e.g. TNF-α have revolutionised treatment and subverted the natural history of the disease.

Spiller et al. identified that in the post-infectious model of IBS, the duration and severity of the insult from an acute presentation of gastroenteritis were significant risk factors in the development of the persistence of IBS symptoms. The up-regulation of the pro-inflammatory state (and its quantification by immunohistochemical means) could feasibly identify predictors for the development of post-infectious IBS.[366] This has obvious ramifications for clinical practice.

Study Methodology

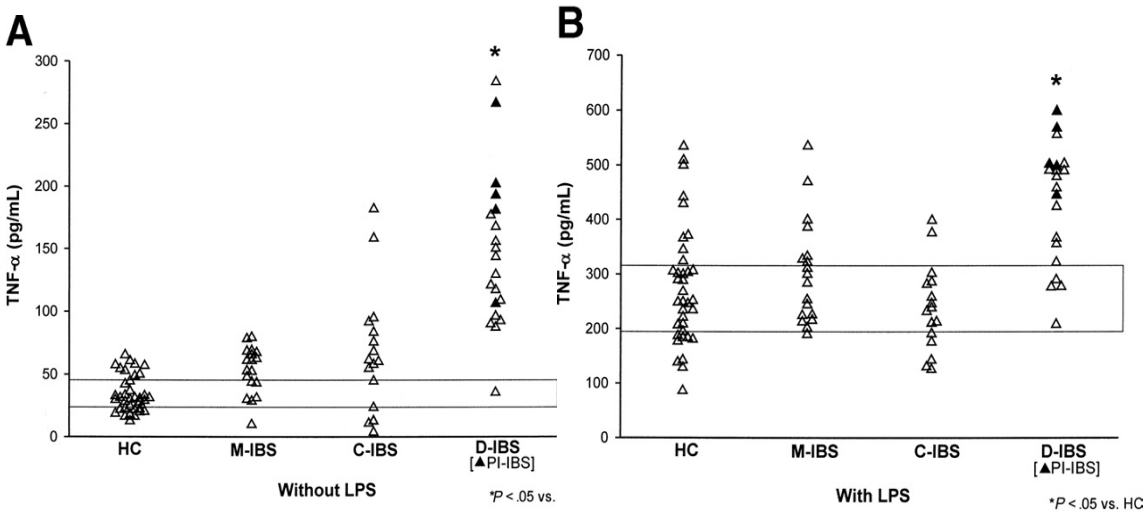
The methodological framework upon which this study was designed came largely from Liebregts et al.[367] This study identified a population of IBS patients using Rome 2 criteria and negative findings at colonoscopy and stool testing. IBS subtyping discriminated into diarrhoea, constipation and mixed-picture phenotypes.

Further inspection of the diarrhoea-predominant patients (numbering 20 in all) identified 5 patients with post-infectious IBS (confirmed with previous positive testing for Campylobacter or Salmonella). Controls were healthy and without previous comorbidity. Psychological assessment comprised the Bowel Disease Questionnaire and the Hospital Anxiety and Depression Scale.

Immunological assessment comprised the harvesting of peripheral blood mononuclear cells (PBMCs) using conventional techniques- blood sampling, centrifugation and suspension in an appropriate medium (RPMI with a cell density of $1x10^6$ cells/ml). The PBMCs were then stimulated with E. coli LPS at a dose of 1ng/ml, and then incubated over a 24-hour period. Cytokine analysis of TNF-α , IL-1β and IL-6 were performed using ELISA techniques. Their data is presented in Figure 4.5.

Comparison of Cytokine Production Between The Studies

Figure 4.5 TNF-α Production from Controls and IBS patients with and without LPS stimulation (Liebgrets et al.)

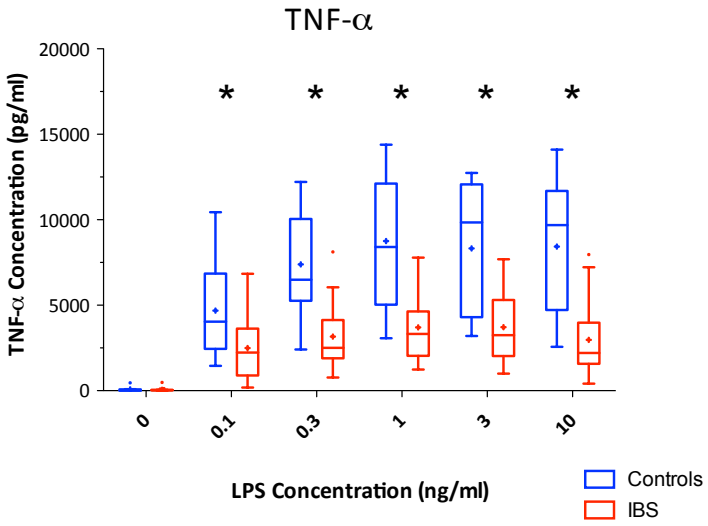


(A) TNF-α production (pg/ml) by PBMCs from healthy controls (HCs) and IBS patients before (baseline) and (B) after stimulation with 1 ng/ml of E coli LPS. Adapted from Liebregts et al.[367]

The boxed area represents the interquartile range of HCs. Under baseline conditions, subjects with D-IBS showed significantly (P<0.05) higher TNF-α levels compared with HCs. LPS co-incubation caused a significant increase of TNF-α in all subjects compared with non-stimulated PBMCs. The LPS-induced TNF-α production was significantly (P =.005) higher in D-IBS patients compared with HCs. No significant differences were observed in M-IBS and C-IBS, whereas PI-IBS showed significantly higher baseline and LPS-stimulated TNF-α levels compared with HCs. Patients with PI-IBS did not differ significantly from patients with D-IBS.

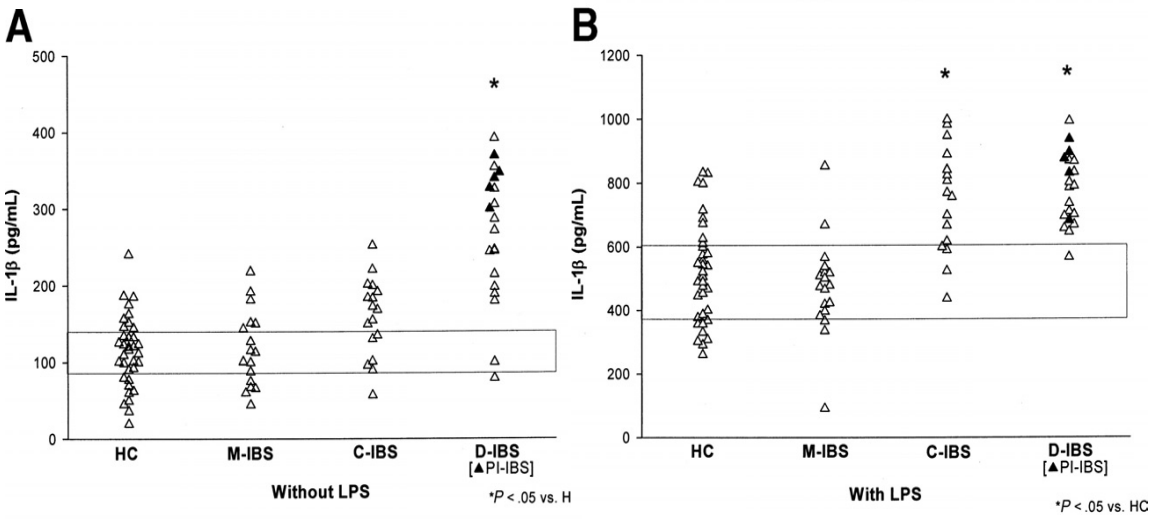
This data compares striking with our own data. The data from our TNF-α analysis shows a significant difference between controls and IBS in the LPS-stimulated dose-response graph (Figure 4.6). When the LPS concentration was 0 pg/ml (i.e. non-stimulated) there was no difference between groups.

Figure 4.6 TNF-α Production from Controls and IBS patients with and without LPS stimulation



The IL-1 β data from the Liebgrets et al. paper is presented in Figure 4.7.

Figure 4.7 IL-1 β Production from Controls and IBS patients with and without LPS stimulation (Liebgrets et al.)

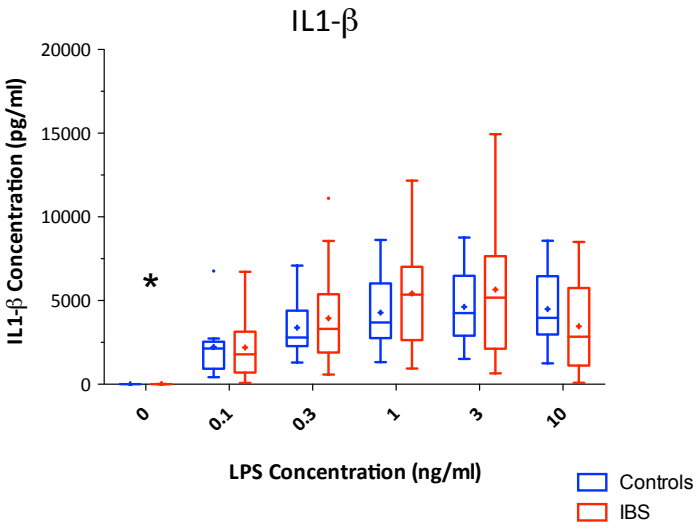


(A) IL-1 β production (pg/ml) by PBMCs from HCs and IBS patients before (baseline) and (B) after stimulation with 1 ng/ml of E coli LPS. Under baseline conditions, subjects with D-IBS showed significantly (P=.004) higher IL-1 β levels compared with HCs. Co-incubation with LPS significantly enhanced IL-1 β production in all subjects compared with baseline. The LPS-induced increase of IL-1 β was significantly higher in DIBS (P = .01) and C-IBS (P = .012) patients compared with HCs,

whereas there were no significant alterations in M-IBS. PI-IBS patients showed significantly (P <.05) higher baseline and LPS-induced IL-1 β levels compared with HCs without a significant difference vs. D-IBS.[367]

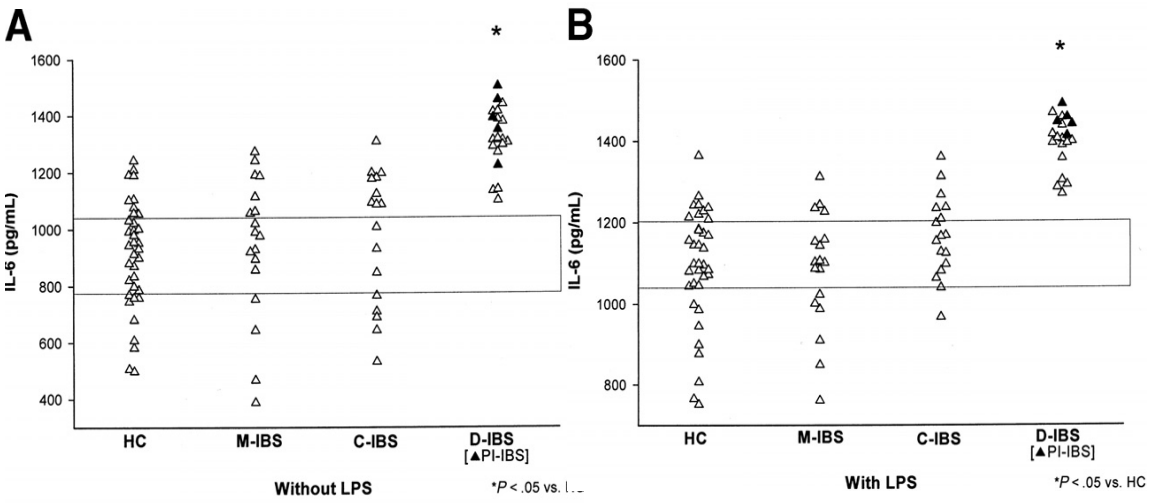
As a comparison, our IL-1 β data is presented in Figure 4.8.

Figure 4.8 IL-1 β Production from Controls and IBS patients with and without LPS stimulation



The IL-6 data from the Liebgrets et al. paper is presented in Figure 4.9.

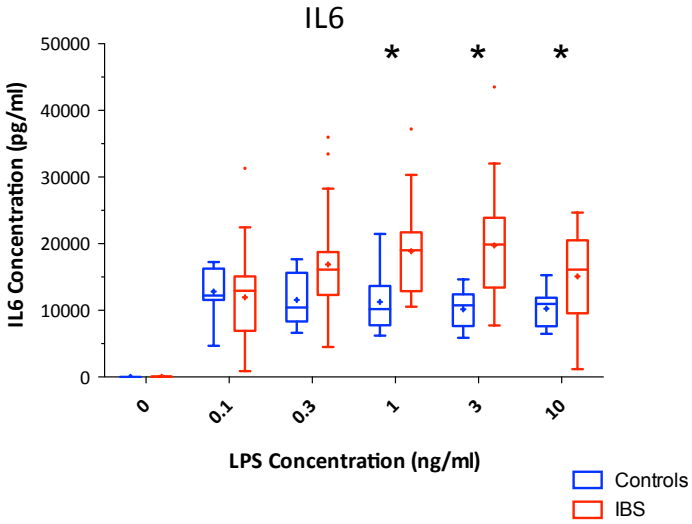
Figure 4.9 IL-6 Production from Controls and IBS patients with and without LPS stimulation (Liebgrets et al.)



(A) IL-6 production (pg/ml) by PBMCs from HCs and IBS patients before (baseline) and (B) after stimulation with 1 ng/ml of E coli LPS. Compared with controls, baseline (P = .005) and LPS-stimulated (P = .011) IL-6 production was significantly higher in D-IBS and (P < .05) PI-IBS patients. There were no significant differences in M-IBS and C-IBS.

As a comparison, our IL-6 data is presented in Figure 4.10.

Figure 4.10 IL-6 Production from Controls and IBS patients with and without LPS stimulation



Side by side comparison of these two studies is fascinating. The original cocktail of TNF-α, IL-1 β and IL-6 was chosen because they represent pro-inflammatory cytokines. Liebregts et al. wanted to test their hypothesis that IBS patients expressed an augmented pro-inflammatory state. Their data showed that the diarrhoea-predominant IBS patients expressed significantly higher levels of all three cytokines both at baseline and after LPS stimulation. This difference was even more prominent in the 5 PI-IBS patients that were isolated from the larger group of 20 diarrhoea-predominant patients.

Our data has contrasting results. TNF- α was expressed more in controls in the LPS-stimulated experiments, IL-1 β did not produce a statistical difference between the two groups, and IL-6 was the only cytokine (of the three) to demonstrate an advantage in favour of the IBS group.

Methodological Differences Between The Two Studies

It is something of a challenge to explain the differences seen in cytokine production between our study and the Liebgrets et al. study. The first point of enquiry lies with the methodology of the two

studies. As far as possible, we tried to emulate the methodology of Liebregts et al. However important (and is some cases deliberate) differences arose.

Inclusion criteria was based on the Rome 2 criteria for both studies, but Liebregts et al. also used colonoscopy and stool analysis to exclude competing diagnoses. In the IBS literature and according to the Rome Foundation, a diagnosis of IBS can be reasonably made when there is adherence and conformity to the Rome criteria in the absence of any red flag symptoms or any other more obvious diagnosis. Traditionally IBS research has not insisted on invasive testing before a subject is considered fit for eligibility to a study. As a result, this was not incorporated into our study design although it was considered but dismissed as adding complexity to the study design, adding unnecessary invasive testing to a group of patients who were already involved in CEP analysis, and increasing the cost and duration of the enterprise.

Patient Recruitment

Patient recruitment was run through a tertiary referral centre in our trial. This meant that the clinical symptom severity of the IBS patients was far greater than one would expect from a community-based or GP-based study. I am sure that this accounts for the marked differences in the psychological assessments seen between the controls and the IBS patients. The Liebregts et al. study recruited from the Outpatient clinics at the Royal Adelaide which is a secondary care facility.

In terms of the numbers recruited, the Liebregts et al. study was far more comprehensive in its scope to attract patients and controls. Fifty-five IBS patients and 36 control patients were included. This is sharply contrasted with our own study, which could only assemble 25 IBS patients and 9 controls. This is a major fault of our study, since it is easy to conclude that the study was underpowered, producing data unrepresentative of either IBS patients or controls. Reasons for why the study had so few numbers stem from the fact that the immunology testing was part of a larger test protocol that included CEP analysis and comprehensive psychological assessment. Furthermore, the intricacy and the complexity of the immunology experiments meant that there were insufficient resources to expand the study sample.

IBS Sub-type Analysis

A further point to make about the recruitment process was that the larger numbers in the Liebregts et al. study allowed them to perform subtype analysis, differentiating cytokine expression between the sub-types. This produced the striking observation that the diarrhoea-predominant IBS immuno-

phenotype, and in particular the post-infectious variant, was pretty much an entity distinct from all other subtypes. The data suggests that an up-regulated immune system in PI-IBS patients could be an important pathogenetic mechanism in maintaining symptoms. To do that in our IBS population of 25 would have produced data of questionable accuracy. Even so, it should be noted that 12 of the 25 (48%) IBS patients in our study were diarrhoea-predominant compared to 20 out of 55 (36%) in the Liebregts et al. study. If diarrhoea-predominance does indeed reflect a pro-inflammatory state then our data should have reflected this.

ELISA vs MSD Plex Technology

The preparation of the PBMCs was conducted using typical laboratory conventions, as outlined previously. Our protocol followed the Liebregts et al. study as closely as possible. The exception to this was the way that the cytokine concentrations were calculated. Their approach was to use an ELISA-based system for the three cytokines. Our approach was to use the MSD Plex multi-array system. This is a commercial, automated system which according to the type of cytokine plates used can produce concurrent readings of multiple cytokines. Its makers, MSD, refer to the term "Electrochemiluminescence Detection" (ECL), which is an awkward commercial term to describe an updated version of the ELISA technology.

In ELISA testing, an unknown solution of antigen is immobilised on a microtitre plate, and then an enzyme-linked antibody is added which effects a colour change. The degree of colour change is proportionate to the concentration of the antigen, in this case a cytokine. Electrochemiluminescence adds the extra dimension of using electron-transfer reactions to excite chemical intermediates that emit light. This degree of light emitted is proportional to the amount of cytokine present.

The benefit of using MSD technology is that multiple cytokines can be assessed in each well (of a 96-well plate). Furthermore it is a much easier and faster system to use- once the 96 well plates are prepared, they are simply slotted into the machine, and the data is then produced by the computer. The downside to them is the exorbitant cost per 96 well plate.

Magnitude of Cytokine Production

The cytokine quantification between the 2 studies is out by an order of magnitude. For a 1ng/ml stimulation of E. coli LPS the mean cytokine reading for TNF-α was 324.8 pg/ml for the Liebregts et al. study and 3701.43 pg/ml in our study. This is a significantly different figure. Perhaps it can be explained by the vagaries of patient selection or even a dilutional error in one or both of the studies.

Another factor would be the method of cytokine quantification, namely the difference in sensitivities between standard ELISA and electrochemiluminescence.

ELISA vs MSD Plex Antigen Sensitivity

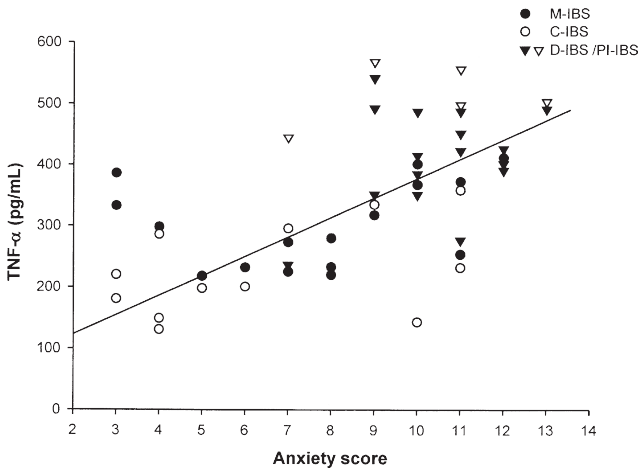
Differences between standard ELISA testing and electrochemiluminescence (ECL) have been studied by various research groups concerned with immunology-related laboratory techniques. A French group compared ELISA to ECL for the detection of a Ricin antigen.[368] They found that the ELISA detected antibody at levels of 400pg/ml of Ricin whereas the newer technique had a limit of detection at 50pg/ml. Furthermore the ELISA assay was completed in 7 hours whereas the ECL completed in 2.5 hours. The disparity of 4.5 hours between completing the two tests with an eminently degradable substrate cannot be ignored. The conclusion was that ECL was 8 times more sensitive that ELISA, 3 times faster and allowed the convenience of using a 96 well plate.

The use of ECL compared to ELISA is sufficient to explain some of the difference in results. Despite the aspiration to emulate the methodology of the Liebregts et al. study, it would have been quite impossible to clone and replicate it without some obfuscation of the original. Certainly the choice between ELISA and ECL was born out of the fact that the superior technology was at our disposal, and that it was a much more efficient technique.

Psychological Parameters

In terms of psychological analysis, the Liebregts et al. study used the Bowel Disease Questionnaire and the Hospital Anxiety and Depression Scale (HAD). The HAD scores showed that IBS patients had significantly higher scores for anxiety (8.4 +/- 3.0) and depression (7.6 +/- 3.6) compared with controls (anxiety, 2.9 +/- 2.7; depression, 2.7 +/- 2.5). This data fits nicely with our own data that demonstrates an increased psychological comorbidity in IBS patients across a spectrum of domains. The Liebregts et al. study reviewed the correlations between cytokine expression and HAD-anxiety states. They observed a positive correlation between LPS-induced TNF-α expression and anxiety ($r = 0.59$, $P < .001$). This data is presented in Figure 4.11. Our own correlation data (Table 3.2.15) demonstrates positive correlations between HAD-anxiety and both IFN-γ and IL-10. TNF-α is negatively correlated to STAI-state anxiety scores and HAD-depression scores.

Figure 4.11 Correlation of E coli LPS-induced TNF-α production and HAD-Anxiety Scores (Liebregts et al.)



Overall, LPS-induced TNF-α release is associated significantly with anxiety scores (r = 0.59, P < .001) in patients with IBS, although it did not reach statistical significance for the IBS subgroups.

Cytokine Dose Response Curves

The Liebregts et al. study used a single dose of E. coli LPS. PBMCs were either stimulated using this dose or not stimulated, left for 24 hours of incubation and then analysed using an ELISA. In practical terms this was a relatively simplistic design. To expand upon their work, our cytokine protocol used a 6 point concentration of LPS to enable dose response curves. This was important since it is relatively easy to see that even at the smaller doses e.g. 0.1 or 0.3 ng/ml of LPS, significant evolution of cytokines from the PBMCS occurs. In the cytokine assays for TNF-α, and IL 2, IL-4, IL-8, IL-10, IL-13 and IFN-γ, LPS stimulation even at an LPS dose of 0.1ng/ml is enough to cause statistically significant separation between the two groups.

At baseline in the non-stimulated samples, only IL-1- β and IL-4 demonstrated significantly different data between control and IBS populations. This contrasts again with the Liebregts et al. study which found statistical difference for their 3 chosen cytokines- TNF-α, IL-1β and IL-6- even at baseline.

Expanding the Cytokine Panel

TNF-α, IL-1β and IL-6 were chosen by Liebregts et al. specifically because of their role as pro-inflammatory cytokines. Since the MSD multi-array technology was available, our study allowed cytokine analysis of a broader range of cytokines, both pro- and anti-inflammatory. The pro-

inflammatory cytokines comprise TNF-α, IFN-γ, IL-1β, IL-2, IL-6, IL-8, and IL-12. The anti-inflammatory cytokines are represented by IL-4, IL-5, IL-10, and IL-13.

Pro-Inflammatory and Anti-Inflammatory Cytokines

Even casual observation of our data shows that there is a mixed pro-inflammatory profile in IBS. Of the pro-inflammatory cytokines, IBS patients produce significantly less TNF-α and IL-2; the data from IFN-γ, IL-1-β and IL-12 is equivocal, yet there is a large production of IL-6 and IL-8. Of the anti-inflammatory cytokines, broadly speaking IBS patients produce less than their healthy counterparts.

With reference to previous immune studies in IBS, and particularly the Liebregts et al. study, our data does not support the hypothesis that IBS patients exist in a state of heightened immune regulation. In order to explain the results that our study has produced, it is important to look at the interaction between the pro-inflammatory immune state and the anti-inflammatory immune state. This has conventionally been seen as the balance between the T_{H1} or T_{H2} systems.

The Immune System and the Brain

The immune system serves as a defensive mechanism to protect against invading pathogens. The balance between the innate and the adaptive systems is influenced by the nature of the pathogen, prior exposure to said pathogen and environmentary pressures. The common language of peptide hormones and neurotransmitters shared between the immune system and the central nervous system has fathered the concept of bidirectional communication between these two systems.[369] Not only can the immune system exert influence on the brain (for example the action of IL-1β on the hypothalamus to produce CRH and ACTH) but also the brain can reciprocate by altering immune function (for example, vagal acetylcholine down-regulates macrophage pro-inflammatory cytokine function).

The classic experiment to show the link between the brain and the immune system was that performed by Ader and Cohen in 1975.[370] They selected a group of rats that had been conditioned with saccharin-infused water and the immunosuppressant cyclophosphamide to produce an illness-induced taste aversion. They injected both conditioned and unconditioned rats with sheep erythrocytes to promote an immune response. Both groups of rats were exposed to the saccharin water (without the immunosuppressant). The conditioned rats demonstrated significantly less antibody production than the unconditioned group, showing that the taste stimulus alone had

triggered the conditioned nervous system to down-regulate the immune response. This landmark study coined the term Psychoneuroimmunology.

The Shifting Balance between TH1 and TH2 Immune Responses

Environmental pressures such as stress can therefore have a profound effect on immune function with preferential stimulation of either the T_{H1} or T_{H2} systems.[371] Animal models have observed that when an animal is presented with an acute stressor, for instance the arrival of an aggressive animal, a T_{H1} mediated immune response is up-regulated. This confers rapid cellular-based immunity to the host who is under threat,[372] thus equipping it to deal with bacterial infection after attack more efficiently.

The Immune System and Stress

The human correlation to such a model, termed the social disruption model, in which an acute predatory threat is present, is fortunately not as common as it used to be. Stress in its various forms has been defined by various investigators.[373] There are thought to be 5 types of stress- *acute time-limited stressors* such as public speaking; *brief naturalistic stressors* that involve the confrontation of a short-term challenge, such as examinations; *stressful event sequences* in which a single, isolated event gives rise to its sequelae (e.g. losing your spouse) but with the individual harbouring the insight or understanding that the stressor will start to disappear; *chronic stressors* which absorb a person's life such that he or she has no idea whether the stress will go away; and finally, *distant stressors* which are traumatic experiences in the past that still cause emotional and cognitive distress (e.g. rape as a child).

Stress-induced Immunosuppression

The relevance of this is that the immune system is influenced by the type and chronicity of the stressor it is subjected to. From a historical perspective, a model of immunosuppression was originally proposed by Selye in 1975 that stated that stress leads to a globally suppressed immune system.[374-376] This gave way to the concept of a biphasic response in which cell-mediated immunity was enhanced by acute stress and dampened by chronic stress. The innate system is much more rapid in its deployment of immune response than the adaptive system, and is less energy-dependent. During the fight or flight response, the host preferentially choses to amplify the innate system to minimise energy expenditure.[377,378]

A year later Marshal et al. proposed that chronic stress causes a dysregulation in the balance of the immune response and a shift in the T_{H1} /T_{H2} axis.[379] Chronic stress leads to a more T_{H2}-weighted environment, such that the individual is more liable to infection and neoplastic disease (reduced T_{H1} activity) and more prone to allergic and autoimmune phenomena (increased T_{H2} activity).

When considering immune changes with reference to the specific type of stressor, a large meta-analysis of over 300 studies looking at immune function and stress in in-vivo studies was performed by Segerstrom et al.[371]

The meta-analysis identified that T_{H1}-mediated immunity is boosted during acute time-limited stress. Among the observations seen were an abrupt increase in the number of natural killer cells and granular lymphocytes in the peripheral blood, a redistribution of T cells towards the skin (as part of a compartmentalisation of immune cells to areas where they may be most effective), an increase in the cytotoxicity of natural killers cells, and a rise in the production of IL-6 and IFN-γ.

Brief naturalistic stressors did not seem to change the population densities of the cells in the immune system, but seemed to have an effect on the functioning of those cells following changes in cytokine production. The net result was a shift to a T_{H2} weighted system (IFN-γ was suppressed; IL-6 and IL-10 were increased; natural killer cell cytotoxicity was diminished).

Stressful event sequences did not produce a uniform pattern of immune changes. This can also be said of distant stressors.

Chronic stressors- such as living with handicap or financial embarrassment- caused a global down-regulation of both T_{H1} and T_{H2} systems.

The Sick Role

Stress-induced changes in an individual's immunophenotype leads to alterations in other areas of one's functioning. This includes alterations of pain sensitivity, psychological state, sleep patterns, appetite and physical activity levels. The complicated reaction to infection or injury, involving immunological, neurological, psychological and philosophical factors promotes the (correct) adoption of the sick role.[380] This is a mechanism designed to confer a biological advantage to an individual[381] and to conserve energy.[382] However this process can become maladaptive, the sick role may continue in the absence of disease, and the symptoms that were stimulated by the initial insult may persist.

Stress and the Hypothalamic-Pituitary-Adrenal Axis

Stress in IBS has been assessed by manipulation of the Hypothalamic-Pituitary-Adrenal axis. Dinan et al. observed an exaggerated release of ACTH and cortisol in IBS patients in response to CRH infusion.[290] Furthermore baseline levels of IL-6 and IL-8 and cortisol were significantly raised, and a positive correlation between ACTH release and IL-6 reached statistical significance.

Taché's group has been pivotal in researching CRF1 signalling pathways in functional disease.[383,384] Stress-induced stimulation of colonic motility, diarrhoea and defaecation with concomitant mast cell degranulation, PGE2 synthesis and increased mucosal permeability can imitated by the injection of CRF in rats.[385]

With regard to the IBS population, stress has an important pathophysiological role. Using a concept similar to Tache's study, Fukodo et al. demonstrated that the biochemical induction of the stress response by intravenous injection of CRH stimulated increased colonic motility, duration of abdominal pain and ACTH response in IBS patients compared to controls, suggesting an exaggerated role of the HPA axis in symptom generation.[386] Sagami proved the opposite point- that the administration of a CRH receptor antagonist produced an improvement in abdominal pain scores, colonic motility and visceral perception after gut stimulation in IBS patients compared to controls.[387]

The importance of CRH is not purely confined to IBS and functional disease- other studies have demonstrated elevated CRH in conditions as diverse as childhood trauma, anxiety states, obsessive compulsive disorders, depressions and post-traumatic stress.[388-390]

Immune Function and Stress

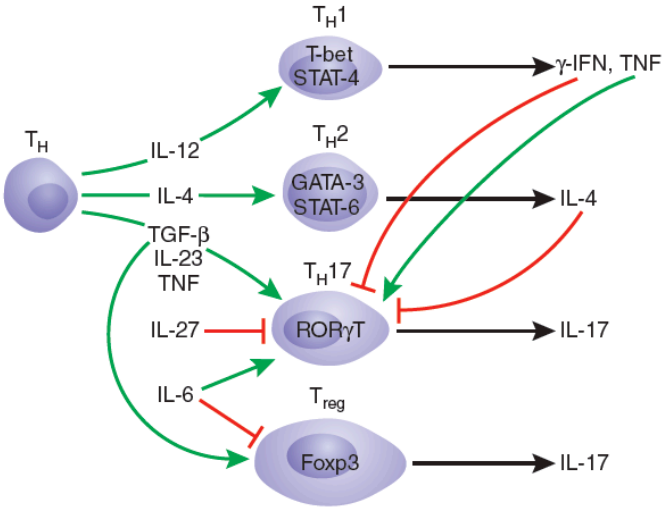
In our study, comprehensive psychological analysis has shown a heavy burden of psychological comorbidity and symptomatology. Increased pro-inflammatory IL-8 levels were positively correlated with unpleasantness of symptoms and somatisation whilst the anti-inflammatory cytokines IL-4 and IL-5 were negatively correlated with the same measures. With increasing symptom severity and psychological comorbidity, our data demonstrated a global immune suppression of both the T_{H1} and T_{H2} pathways. As reflected by Segerstrom et al. this would be consistent with the immune changes seen in a chronic stress environment.[371]

T_{H17} Pathway

The two cytokines that were increased in the IBS group in our data were IL-6 and IL-8. IL-6 promotes T cell differentiation and drives the maturation of the T_{Reg} and T_{H17} cell lines. It is possible that the over-expression of IL-6 is driving a T_{H17} pathway.

T_{H17} is a recently discovered subset of T helper cells, sitting beside the classical T_{H1} and T_{H2} pathways. It was first proposed in 2000 by Infante-Duarte et al[391] who demonstrated a novel non-T_{H1} non-T_{H2} CD4+ cell which evolved IL-17 rather than IFN-γ or Il-4. Subsequent studies demonstrated that the T_{H17} cell line develops in response to TGF-β, IL-6, IL-1 and IL-21, and that T_{H17} cells also produce IL-21 and IL-22.[392]. The T_{H17} pathway is seen in Figure 4.12.

Figure 4.12 The T_{H17} Pathway



Adapted from A brief history of T_{H17}, the first major revision in the T_{H1}/T_{H2} hypothesis of T cell-mediated tissue damage by Steinman, L and Steinman, L[393]

The T_{H17} subset serves to target Gram-negative bacteria, fungi and certain protozoa. T_{H17} over-expression has been associated with autoimmune and inflammatory diseases. These include rheumatoid arthritis, SLE, autoimmune encephalitis and bronchial asthma.[394-396]

T_{H17} cells facilitate neutrophil recruitment, activation and migration and are thought to be an important part of mucosal defence.[396] They predominate in mucosal and epithelial junctions, for example the GI tract.

An increased T_{H17} profile as suggested by our data would stimulate mucosal immunity and a pro-inflammatory cytokine profile. T_{H17} differentiation is at its most potent when both T_{H1} and T_{H2} effector functions are suppressed.[395,397,398] Furthermore an enhanced T_{H17} response causes an upregulation in the production of IL-6, IL-8 and metalloproteinases (MMPs) in major target cells such as sub-epithelial myofibroblasts.[399]

Conclusion

Our cytokine data demonstrates T_{H1} and T_{H2} immune suppression with elevated IL-6 and IL-8 levels in IBS. This is consistent with the immunophenotype of chronic stress, and perhaps represents a shift to a T_{H17} profile. The follow-up to this study would be to reconstruct the protocol with specific reference to quantifying Il-17, IL-23 and TGF-β since this would give substance to the novel hypothesis of a T_{H17} profile in IBS.

FINAL CONCLUSION

This study has used 4 modalities of testing to separate IBS patients from their control counterparts. The psychological data has been consistent with the established literature about the depth of psychological comorbidity found in IBS patients. It has identified certain character traits that may be more common in this condition, e.g. neuroticism and introversion. The clinical challenge is being able to tease apart the psychological comorbidity associated with IBS from a primary psychological process that shares the outward manifestation of gastrointestinal symptomatology. Needless to say the prompt identification of psychological comorbidity is imperative for the appropriate management of the patient.

The use of electrical stimulation of the rectum did not discriminate between the two groups in terms of pain threshold until the final day of testing. This is in contrast to previous studies that differentiated patients and controls using balloon distension paradigms. Perhaps by removing the emotional context of the stimulus (i.e. a rapidly inflating and deflating balloon in the rectum with the incipient sensation of impending defaecation), renders the hypersensitive rectum normosensitive by minimising subjective sensory perception. Conversely, the disinhibition of a control subject through habituation produces an increase in pain threshold.

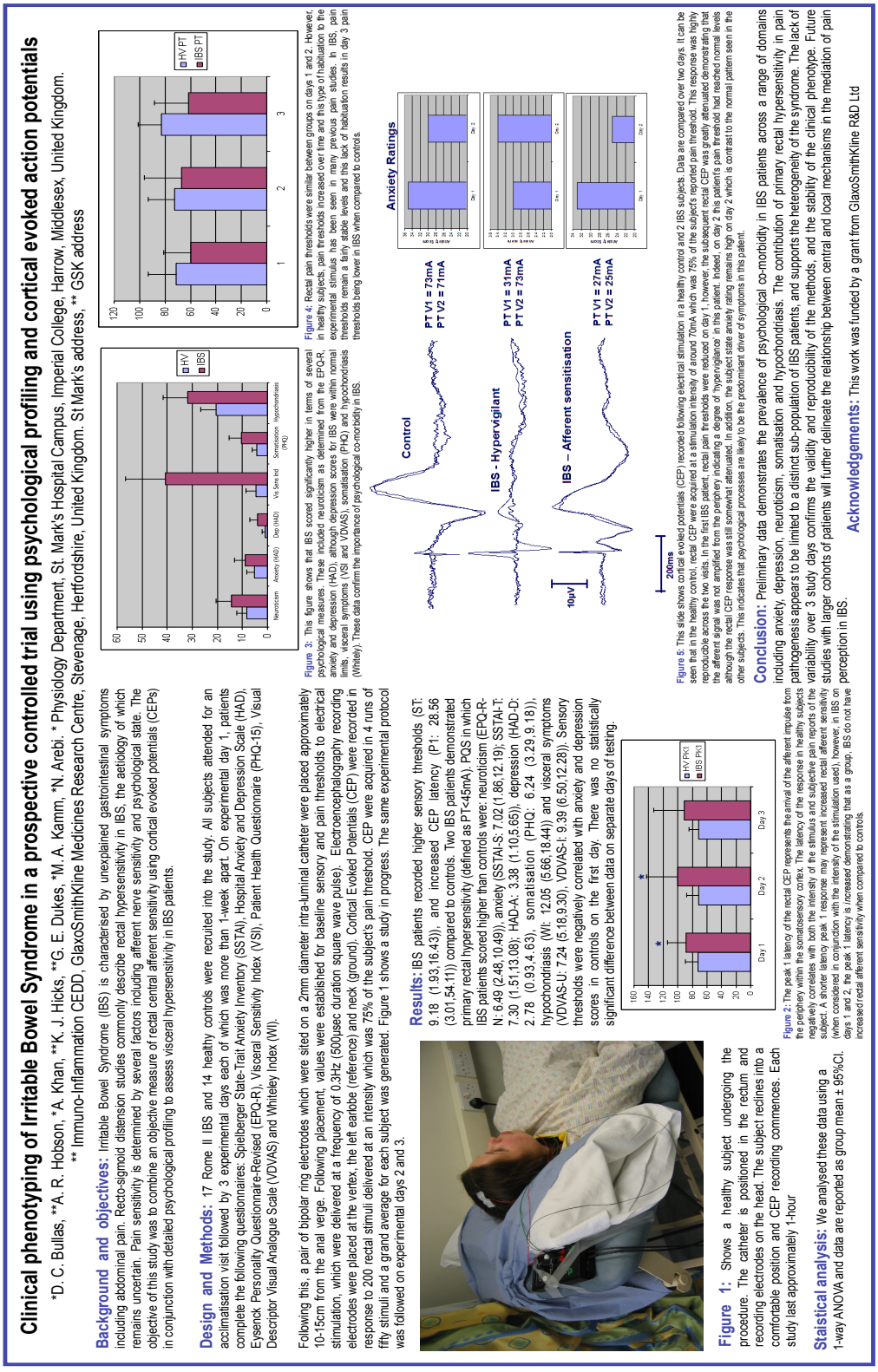
CEP analysis in conjunction with anorectal sensitivity produced 4 sub-groups of IBS patients- those with normal CEPs and pain threshold, those with afferent hyper or hyposensitivity, and those who were hypervigilant. Further work, and much larger studies, will need to assess whether this has a useful clinical context. The identification of sub-groups potentially offers targeted therapy to the individual.

The immunology of IBS has become a popular area of research over the last decade. Many studies have demonstrated a pro-inflammatory state in IBS, either by quantifying cytokine expression in peripheral blood cells or colonic mucosal samples. Our own data did not support this, instead suggesting that other pathways, namely the T_{H17} pathway, may be important. Further work needs to agree on a uniform methodology for investigating cytokine expression in IBS, to look at novel pathways beyond the established T_{H1} /T_{H2} axis, to expand the samples sizes, and to replicate previous studies to make sure that the differences seen in earlier work are universal and reproducible.

APPENDIX

(Continued overleaf)

Clinical phenotyping of Irritable Bowel Syndrome in a prospective controlled trial using psychological profiling and cortical evoked action potentials



Clinical phenotyping of Irritable Bowel Syndrome in a prospective controlled trial using psychological profiling and cortical evoked action potentials

*D. C. Bullas, **A. R. Hobson, *A. Khan, **K. J. Hicks, **G. E. Dukes, *M. A. Kamm, *N. Arebi. * Physiology Department, St. Mark's Hospital Campus, Imperial College, Harrow, Middlesex, United Kingdom.
** Immuno-Inflammation CEDD, GlaxoSmithKline Medicines Research Centre, Stevenage, Hertfordshire, United Kingdom. St Mark's address, ** GSK address

Background and objectives: Irritable Bowel Syndrome (IBS) is characterised by unexplained gastrointestinal symptoms including abdominal pain. Recto-sigmoid distension studies commonly describe rectal hypersensitivity in IBS, the aetiology of which remains uncertain. Pain sensitivity is determined by several factors including afferent nerve sensitivity and psychological state. The objective of this study was to combine an objective measure of rectal central afferent sensitivity using cortical evoked potentials (CEPs) in conjunction with detailed psychological profiling to assess visceral hypersensitivity in IBS patients.

Design and Methods: 17 Rome II IBS and 14 healthy controls were recruited into the study. All subjects attended for an acclimatisation visit followed by 3 experimental days each of which was more than 1-week apart. On experimental day 1, patients complete the following questionnaires: Spielberger State-Trait Anxiety Inventory (SSTAI), Hospital Anxiety and Depression Scale (HAD), Eysenck Personality Questionnaire-Revised (EPQ-R), Visceral Sensitivity Index (VSI), Patient Health Questionnaire (PHQ-15), Visual Descriptor Visual Analogue Scale (VDVAS) and Whiteley Index (WI).

Following this, a pair of bipolar ring electrodes which were sited on a 2mm diameter intra-luminal catheter were placed approximately 10-15cm from the anal verge. Following placement, values were established for baseline sensory and pain thresholds to electrical stimulation, which were delivered at a frequency of 0.3Hz (500μsec duration square wave pulse). Electroencephalography recording electrodes were placed at the vertex, the left earlobe (reference) and neck (ground). Cortical Evoked Potentials (CEP) were recorded in response to 200 rectal stimuli delivered at an intensity which was 75% of the subject's pain threshold. CEP were acquired in 4 runs of fifty stimuli and a grand average for each subject was generated. Figure 1 shows a study in progress. The same experimental protocol was followed on experimental days 2 and 3.



Figure 1: Shows a healthy subject undergoing the procedure. The catheter is positioned in the rectum and recording electrodes on the head. The subject reclines into a comfortable position and CEP recording commences. Each study last approximately 1-hour

Staistical analysis: We analysed these data using a 1-way ANOVA and data are reported as group mean ± 95%CI.

Results: IBS patients recorded higher sensory thresholds (ST: 9.18 (1.93,16.43)), and increased CEP latency (P1: 28.56 (3.01,54.11)) compared to controls. Two IBS patients demonstrated primary rectal hypersensitivity (defined as PT<45mA). PQS in which IBS patients scored higher than controls were: neuroticism (EPQ-R-N: 6.49 (2.48,10.49)), anxiety (SSTAI-S: 7.02 (1.86,12.19); SSTAI-T: 7.30 (1.51,13.08); HAD-A: 3.38 (1.10,5.65)), depression (HAD-D: 2.78 (0.93,4.63)), somatisation (PHQ: 6.24 (3.29,9.18)), hypochondriasis (WI: 12.05 (5.66,18.44)) and visceral symptoms (VDVAS-U: 7.24 (5.18,9.30), VDVAS-I: 9.39 (6.50,12.28)). Sensory thresholds were negatively correlated with anxiety and depression scores in controls on the first day. There was no statistically significant difference between data on separate days of testing.

Figure 2: The peak 1 latency of the rectal CEP represents the arrival of the afferent impulse from the periphery within the somatosensory cortex. The latency of the response in healthy subjects negatively correlates with both the intensity of the stimulus and subjective pain reports of the subject. A shorter latency peak 1 response may represent increased rectal afferent sensitivity (when considered in conjunction with the intensity of the stimulation used), however, in IBS on days 1 and 2, the peak 1 latency is *increased* demonstrating that as a group, IBS do not have increased rectal afferent sensitivity when compared to controls.

Figure 3: This figure shows that IBS scored significantly higher in terms of several psychological measures. These included neuroticism as determined from the EPQ-R, anxiety and depression (HAD), although depression scores for IBS were within normal limits, visceral symptoms (VSI and VDVAS), somatisation (PHQ) and hypochondriasis (Whiteley). These data confirm the importance of psychological co-morbidity in IBS.

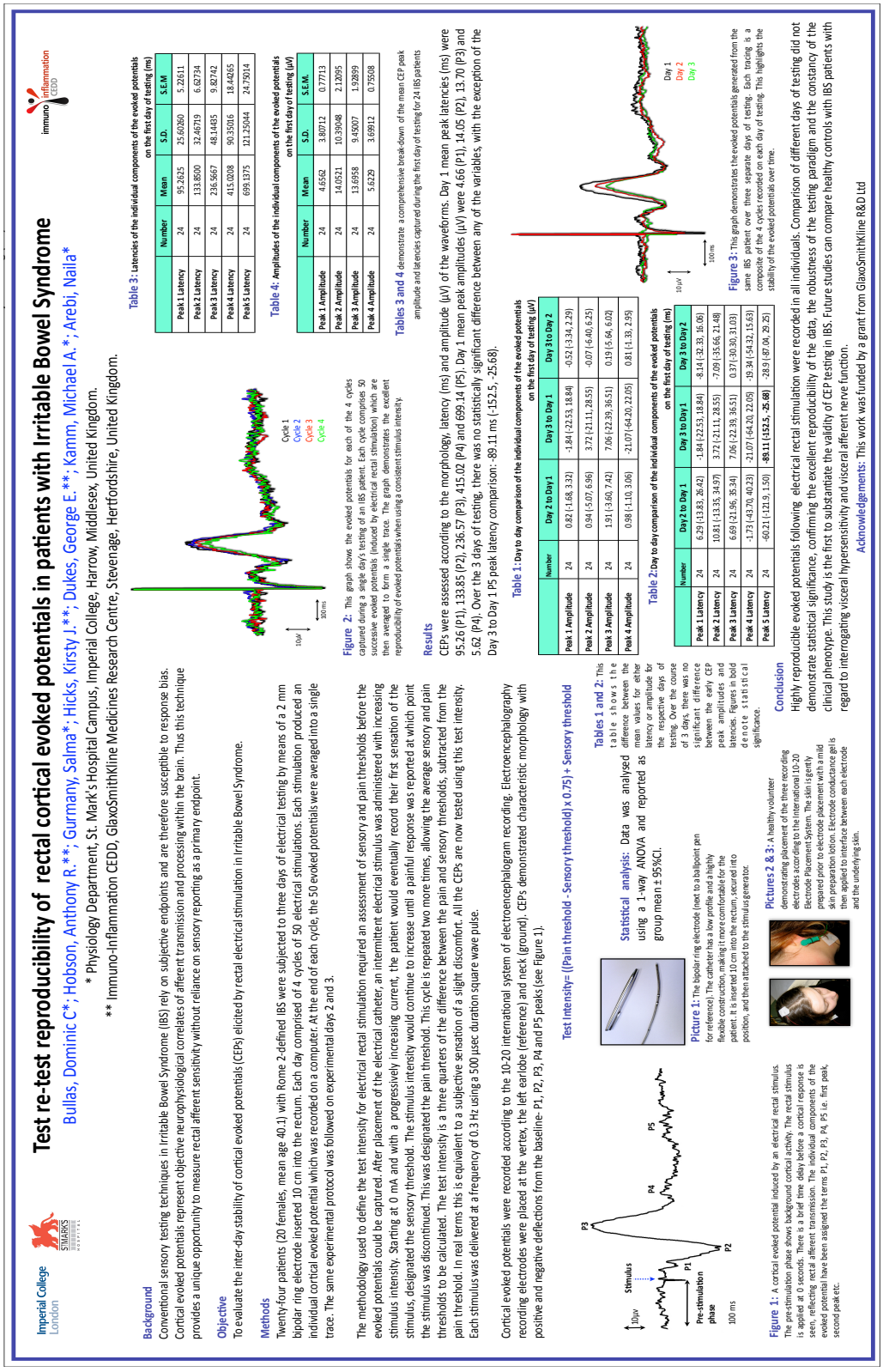
Figure 4: Rectal pain thresholds were similar between groups on days 1 and 2. However, in healthy subjects, pain thresholds increased over time and this type of habituation to the experimental stimulus has been seen in many previous pain studies. In IBS, pain thresholds remain a fairly stable levels and this lack of habituation results in day 3 pain thresholds being lower in IBS when compared to controls.

Figure 5: This slide shows cortical evoked potentials (CEP) recorded following electrical stimulation in a healthy control and 2 IBS subjects. Data are compared over two days. It can be seen that in the healthy control, rectal CEP were acquired at a stimulation intensity of around 70mA which was 75% of the subject's reported pain threshold. This response was highly reproducible across the two visits. In the first IBS patient, rectal pain thresholds were reduced on day 1, however, the subsequent rectal CEP was greatly attenuated demonstrating that the afferent signal was not amplified from the periphery indicating a degree of 'hypervigilance' in this patient. Indeed, on day 2 this patient's pain threshold had reached normal levels although the rectal CEP response was still somewhat attenuated. In addition, the subject state anxiety rating remains high on day 2 which is contrast to the normal pattern seen in the other subjects. This indicates that psychological processes are likely to be the predominant driver of symptoms in this patient.

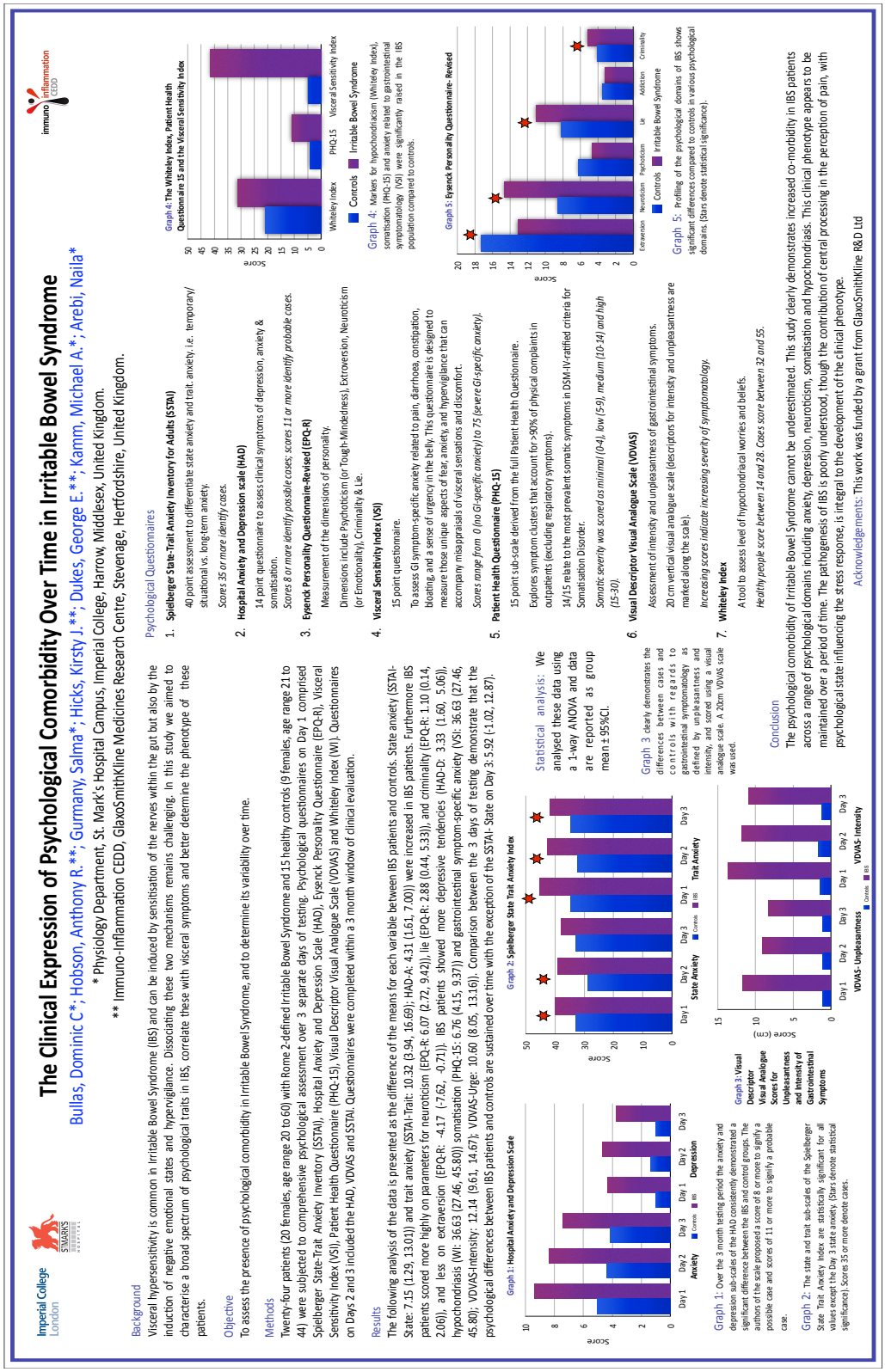
Conclusion: Preliminary data demonstrates the prevalence of psychological co-morbidity in IBS patients across a range of domains including anxiety, depression, neuroticism, somatisation and hypochondriasis. The contribution of primary rectal hypersensitivity in pain pathogenesis appears to be limited to a distinct sub-population of IBS patients, and supports the heterogeneity of the syndrome. The lack of variability over 3 study days confirms the validity and reproducibility of the methods, and the stability of the clinical phenotype. Future studies with larger cohorts of patients will further delineate the relationship between central and local mechanisms in the mediation of pain perception in IBS.

Acknowledgements: This work was funded by a grant from GlaxoSmithKline R&D Ltd

Test re-test reproducibility of rectal cortical evoked potentials in patients with Irritable Bowel Syndrome



The clinical expression of psychological comorbidity over time in Irritable Bowel Syndrome



The Clinical Expression of Psychological Comorbidity Over Time in Irritable Bowel Syndrome

Bullas, Dominic C*; Hobson, Anthony R.**; Gurmany, Salma*; Hicks, Kirsty J.**; Dukes, George E.**; Kamm, Michael A.*; Arebi, Naila*

* Physiology Department, St. Mark's Hospital Campus, Imperial College, Harrow, Middlesex, United Kingdom.

** Immuno-Inflammation CEDD, GlaxoSmithKline Medicines Research Centre, Stevenage, Hertfordshire, United Kingdom.

Background

Visceral hypersensitivity is common in Irritable Bowel Syndrome (IBS) and can be induced by sensitisation of the nerves within the gut but also by the induction of negative emotional states and hypervigilance. Dissociating these two mechanisms remains challenging. In this study we aimed to characterise a broad spectrum of psychological traits in IBS, correlate these with visceral symptoms and better determine the phenotype of these patients.

Objective

To assess the presence of psychological comorbidity in Irritable Bowel Syndrome, and to determine its variability over time.

Methods

Twenty-four patients (20 females, age range 20 to 60) with Rome 2-defined Irritable Bowel Syndrome and 15 healthy controls (9 females, age range 21 to 44) were subjected to comprehensive psychological assessment over 3 separate days of testing. Psychological questionnaires on Day 1 comprised Spielberger State-Trait Anxiety Inventory (SSTAI), Hospital Anxiety and Depression Scale (HAD), Eysenck Personality Questionnaire (EPQ-R), Visceral Sensitivity Index (VSI), Patient Health Questionnaire (PHQ-15), Visual Descriptor Visual Analogue Scale (VDVAS) and Whiteley Index (WI). Questionnaires on Days 2 and 3 included the HAD, VDVAS and SSTAI. Questionnaires were completed within a 3 month window of clinical evaluation.

Results

The following analysis of the data is presented as the difference of the means for each variable between IBS patients and controls. State anxiety (SSTAI-State: 7.15 (1.29, 13.01)) and trait anxiety (SSTAI-Trait: 10.32 (3.94, 16.69); HAD-A: 4.31 (1.61, 7.00)) were increased in IBS patients. Furthermore IBS patients scored more highly on parameters for neuroticism (EPQ-R: 6.07 (2.72, 9.42)), lie (EPQ-R: 2.88 (0.44, 5.33)), and criminality (EPQ-R: 1.10 (0.14, 2.06)), and less on extraversion (EPQ-R: -4.17 (-7.62, -0.71)). IBS patients showed more depressive tendencies (HAD-D: 3.33 (1.60, 5.06)), hypochondriasis (WI: 36.63 (27.46, 45.80)) somatisation (PHQ-15: 6.76 (4.15, 9.37)) and gastrointestinal symptom-specific anxiety (VSI: 36.63 (27.46, 45.80)); VDVAS-Intensity: 12.14 (9.61, 14.67); VDVAS-Urge: 10.60 (8.05, 13.16)). Comparison between the 3 days of testing demonstrate that the psychological differences between IBS patients and controls are sustained over time with the exception of the SSTAI- State on Day 3: 5.92 (-1.02, 12.87).

Graph 1: Hospital Anxiety and Depression Scale

Graph 1: Over the 3 month testing period the anxiety and depression sub-scales of the HAD consistently demonstrated a significant difference between the IBS and control groups. The authors of the scale proposed a score of 8 or more to signify a possible case and scores of 11 or more to signify a probable case.

Graph 2: Spielberger State Trait Anxiety Index

Graph 2: The state and trait sub-scales of the Spielberger State Trait Anxiety Index are statistically significant for all values except the Day 3 state anxiety. (Stars denote statistical significance). Scores 35 or more denote cases.

Graph 3: Visual Descriptor Visual Analogue Scores for Unpleasantness and Intensity of Gastrointestinal Symptoms

Psychological Questionnaires

1. **Spielberger State-Trait Anxiety Inventory for Adults (SSTAI)**
   40 point assessment to differentiate state anxiety and trait. anxiety. i.e. temporary/ situational vs. long-term anxiety.
   Scores 35 or more identify cases.
2. **Hospital Anxiety and Depression scale (HAD)**
   14 point questionnaire to assess clinical symptoms of depression, anxiety & somatisation.
   Scores 8 or more identify possible cases; scores 11 or more identify probable cases.
3. **Eysenck Personality Questionnaire-Revised (EPQ-R)**
   Measurement of the dimensions of personality.
   Dimensions include Psychoticism (or Tough-Mindedness), Extroversion, Neuroticism (or Emotionality), Criminality & Lie.
4. **Visceral Sensitivity Index (VSI)**
   15 point questionnaire.
   To assess GI symptom-specific anxiety related to pain, diarrhoea, constipation, bloating, and a sense of urgency in the belly. This questionnaire is designed to measure those unique aspects of fear, anxiety, and hypervigilance that can accompany misappraisals of visceral sensations and discomfort.
   Scores range from 0 (no GI-specific anxiety) to 75 (severe GI-specific anxiety).
5. **Patient Health Questionnaire (PHQ-15)**
   15 point sub-scale derived from the full Patient Health Questionnaire.
   Explores symptom clusters that account for >90% of physical complaints in outpatients (excluding respiratory symptoms).
   14/15 relate to the most prevalent somatic symptoms in DSM-IV-ratified criteria for Somatisation Disorder.
   Somatic severity was scored as minimal (0-4), low (5-9), medium (10-14) and high (15-30).
6. **Visual Descriptor Visual Analogue Scale (VDVAS)**
   Assessment of intensity and unpleasantness of gastrointestinal symptoms.
   20 cm vertical visual analogue scale (descriptors for intensity and unpleasantness are marked along the scale).
   Increasing scores indicate increasing severity of symptomatology.
7. **Whiteley Index**
   A tool to assess level of hypochondriacal worries and beliefs.
   Healthy people score between 14 and 28. Cases score between 32 and 55.

Statistical analysis:

We analysed these data using a 1-way ANOVA and data are reported as group mean ± 95%CI.

Graph 3 clearly demonstrates the differences between cases and controls with regards to gastrointestinal symptomatology as defined by unpleasantness and intensity, and scored using a visual analogue scale. A 20cm VDVAS scale was used.

Graph 4: The Whiteley Index, Patient Health Questionnaire 15 and the Visceral Sensitivity Index

Graph 4: Markers for hypochondriacism (Whiteley Index), somatisation (PHQ-15) and anxiety related to gastrointestinal symptomatology (VSI) were significantly raised in the IBS population compared to controls.

Graph 5: Eysenck Personality Questionnaire- Revised

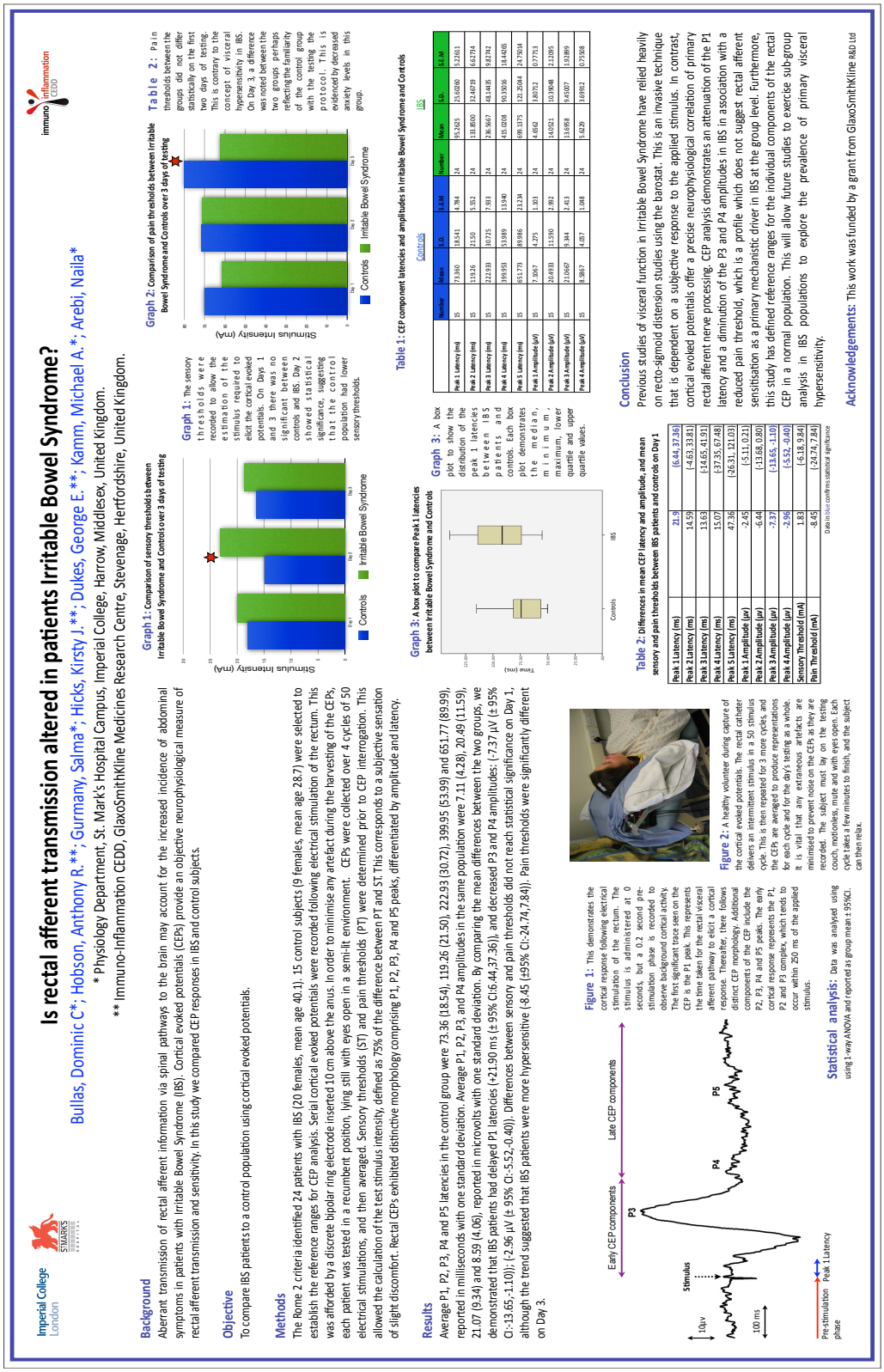
Graph 5: Profiling of the psychological domains of IBS shows significant differences compared to controls in various psychological domains. (Stars denote statistical significance).

Conclusion

The psychological comorbidity of Irritable Bowel Syndrome cannot be underestimated. This study clearly demonstrates increased co-morbidity in IBS patients across a range of psychological domains including anxiety, depression, neuroticism, somatisation and hypochondriasis. This clinical phenotype appears to be maintained over a period of time. The pathogenesis of IBS is poorly understood, though the contribution of central processing in the perception of pain, with psychological state influencing the stress response, is integral to the development of the clinical phenotype.

Acknowledgements: This work was funded by a grant from GlaxoSmithKline R&D Ltd

Is rectal afferent transmission altered in patients with Irritable Bowel Syndrome?



Is rectal afferent transmission altered in patients Irritable Bowel Syndrome?

Bullas, Dominic C*; Hobson, Anthony R. **; Gurmany, Salma*; Hicks, Kirsty J.**; Dukes, George E.**; Kamm, Michael A.*; Arebi, Naila*

* Physiology Department, St. Mark's Hospital Campus, Imperial College, Harrow, Middlesex, United Kingdom.

** Immuno-Inflammation CEDD, GlaxoSmithKline Medicines Research Centre, Stevenage, Hertfordshire, United Kingdom.

Background

Aberrant transmission of rectal afferent information via spinal pathways to the brain may account for the increased incidence of abdominal symptoms in patients with Irritable Bowel Syndrome (IBS). Cortical evoked potentials (CEPs) provide an objective neurophysiological measure of rectal afferent transmission and sensitivity. In this study we compared CEP responses in IBS and control subjects.

Objective

To compare IBS patients to a control population using cortical evoked potentials.

Methods

The Rome 2 criteria identified 24 patients with IBS (20 females, mean age 40.1). 15 control subjects (9 females, mean age 28.7) were selected to establish the reference ranges for CEP analysis. Serial cortical evoked potentials were recorded following electrical stimulation of the rectum. This was afforded by a discrete bipolar ring electrode inserted 10 cm above the anus. In order to minimise any artefact during the harvesting of the CEPs, each patient was tested in a recumbent position, lying still with eyes open in a semi-lit environment. CEPs were collected over 4 cycles of 50 electrical stimulations, and then averaged. Sensory thresholds (ST) and pain thresholds (PT) were determined prior to CEP interrogation. This allowed the calculation of the test stimulus intensity, defined as 75% of the difference between PT and ST. This corresponds to a subjective sensation of slight discomfort. Rectal CEPs exhibited distinctive morphology comprising P1, P2, P3, P4 and P5 peaks, differentiated by amplitude and latency.

Results

Average P1, P2, P3, P4 and P5 latencies in the control group were 73.36 (18.54), 119.26 (21.50), 222.93 (30.72), 399.95 (53.99) and 651.77 (89.99), reported in milliseconds with one standard deviation. Average P1, P2, P3, and P4 amplitudes in the same population were 7.11 (4.28), 20.49 (11.59), 21.07 (9.34) and 8.59 (4.06), reported in microvolts with one standard deviation. By comparing the mean differences between the two groups, we demonstrated that IBS patients had delayed P1 latencies (+21.90 ms (± 95% CI:6.44,37.36)) and decreased P3 and P4 amplitudes: (-7.37 µV (± 95% CI:-13.65,-1.10)); (-2.96 µV (± 95% CI:-5.52,-0.40)). Differences between sensory and pain thresholds did not reach statistical significance on Day 1, although the trend suggested that IBS patients were more hypersensitive (-8.45 (±95% CI:-24.74,7.84)). Pain thresholds were significantly different on Day 3.

Graph 1: Comparison of sensory thresholds between Irritable Bowel Syndrome and Controls over 3 days of testing

Graph 1: The sensory thresholds were recorded to allow the estimation of the stimulus required to elicit the cortical evoked potentials. On Days 1 and 3 there was no significant between controls and IBS. Day 2 showed statistical significance, suggesting that the control population had lower sensory thresholds.

Graph 2: Comparison of pain thresholds between Irritable Bowel Syndrome and Controls over 3 days of testing

Table 2: Pain thresholds between the groups did not differ statistically on the first two days of testing. This is contrary to the concept of visceral hypersensitivity in IBS. On Day 3, a difference was noted between the two groups perhaps reflecting the familiarity of the control group with the testing the protocol. This is evidenced by decreased anxiety levels in this group.

Graph 3: A box plot to compare Peak 1 latencies between Irritable Bowel Syndrome and Controls

Graph 3: A box plot to show the distribution of the peak 1 latencies between IBS patients and controls. Each box plot demonstrates the median, minimum, maximum, lower quartile and upper quartile values.

Table 1: CEP component latencies and amplitudes in Irritable Bowel Syndrome and Controls

Controls

	Number	Mean	S.D.	S.E.M.
Peak 1 Latency (ms)	15	73.360	18.541	4.784
Peak 2 Latency (ms)	15	119.26	21.50	5.552
Peak 3 Latency (ms)	15	222.93	30.715	7.931
Peak 4 Latency (ms)	15	399.95	53.989	13.940
Peak 5 Latency (ms)	15	651.770	89.986	23.234
Peak 1 Amplitude (µV)	15	7.1067	4.275	1.103
Peak 2 Amplitude (µV)	15	20.4933	11.590	2.992
Peak 3 Amplitude (µV)	15	21.0667	9.344	2.413
Peak 4 Amplitude (µV)	15	8.5867	4.057	1.048

IBS

	Number	Mean	S.D.	S.E.M.
Peak 1 Latency (ms)	24	95.2625	25.60260	5.22611
Peak 2 Latency (ms)	24	133.8500	32.46779	6.62734
Peak 3 Latency (ms)	24	236.5667	45.14436	9.21742
Peak 4 Latency (ms)	24	415.0208	90.23036	18.42426
Peak 5 Latency (ms)	24	698.1375	132.25944	26.99034
Peak 1 Amplitude (µV)	24	4.4042	3.80712	0.77713
Peak 2 Amplitude (µV)	24	14.0321	10.19048	2.12095
Peak 3 Amplitude (µV)	24	13.6958	9.45307	1.92989
Peak 4 Amplitude (µV)	24	5.4229	3.69962	0.75508

Figure 1: This demonstrates the cortical response following electrical stimulation of the rectum. The stimulus is administered at 0 seconds, but a 0.2 second pre-stimulation phase is recorded to observe background cortical activity. The first significant trace seen on the CEP is the P1 peak. This represents the time taken for the rectal visceral afferent pathway to elicit a cortical response. Thereafter, there follows distinct CEP morphology. Additional components of the CEP include the P2, P3, P4 and P5 peaks. The early cortical response represents the P1, P2 and P3 complex, which tends to occur within 250 ms of the applied stimulus.

Figure 2: A healthy volunteer during capture of the cortical evoked potentials. The rectal catheter delivers an intermittent stimulus in a 50 stimulus cycle. This is then repeated for 3 more cycles, and the CEPs are averaged to produce representations for each cycle and for the day's testing as a whole. It is vital that any extraneous artefacts are minimised to prevent noise on the CEPs as they are recorded. The subject must lay on the testing couch, motionless, mute and with eyes open. Each cycle takes a few minutes to finish, and the subject can then relax.

Table 2: Differences in mean CEP latency and amplitude, and mean sensory and pain thresholds between IBS patients and controls on Day 1

Peak 1 Latency (ms)	21.9	(6.44, 37.36)
Peak 2 Latency (ms)	14.59	(-4.63, 33.81)
Peak 3 Latency (ms)	13.63	(-14.65, 41.91)
Peak 4 Latency (ms)	15.07	(-37.35, 67.48)
Peak 5 Latency (ms)	47.36	(-26.31, 121.03)
Peak 1 Amplitude (µv)	-2.45	(-5.11, 0.21)
Peak 2 Amplitude (µv)	-6.44	(-13.68, 0.80)
Peak 3 Amplitude (µv)	-7.37	(-13.65, -1.10)
Peak 4 Amplitude (µv)	-2.96	(-5.52, -0.40)
Sensory Threshold (mA)	1.83	(-6.18, 9.84)
Pain Threshold (mA)	-8.45	(-24.74, 7.84)

Data in blue confirms statistical significance

Statistical analysis:

Data was analysed using using 1-way ANOVA and reported as group mean ± 95%CI.

Conclusion

Previous studies of visceral function in Irritable Bowel Syndrome have relied heavily on recto-sigmoid distension studies using the barostat. This is an invasive technique that is dependent on a subjective response to the applied stimulus. In contrast, cortical evoked potentials offer a precise neurophysiological correlation of primary rectal afferent nerve processing. CEP analysis demonstrates an attenuation of the P1 latency and a diminution of the P3 and P4 amplitudes in IBS in association with a reduced pain threshold, which is a profile which does not suggest rectal afferent sensitisation as a primary mechanistic driver in IBS at the group level. Furthermore, this study has defined reference ranges for the individual components of the rectal CEP in a normal population. This will allow future studies to exercise sub-group analysis in IBS populations to explore the prevalence of primary visceral hypersensitivity.

Acknowledgements: This work was funded by a grant from GlaxoSmithKline R&D Ltd

Neurophysiological assessment of rectal afferent sensitivity in patients with Irritable Bowel Syndrome defines distinct clinical phenotypes.

Bullas, Dominic C*; Hobson, Anthony R.**; Gurmany, Salma*; Hicks, Kirsty J.**; Dukes, George E.**; Kamm, Michael A.*; Arebi, Naila*

* Physiology Department, St. Mark's Hospital Campus, Imperial College, Harrow, Middlesex, United Kingdom.

** Immuno-Inflammation CEDD, GlaxoSmithKline Medicines Research Centre, Stevenage, Hertfordshire, United Kingdom.

Background

Patients with Irritable Bowel Syndrome (IBS) report symptoms of abdominal pain in the absence of any discernible gut pathophysiology. Pain amplification can occur via either peripheral sensitisation of gut nerves, central sensitisation of spinal nerves or via top down abnormalities in endogenous pain modulation. Identification of the precise mechanism responsible for the clinical manifestation of symptoms remains a long-term goal and in this study we used cortical evoked potentials (CEPs) to examine if this technique could identify specific phenotypes within an apparently clinically homogenous IBS population.

Objective

To define the role of primary rectal hypersensitivity and psychological factors in IBS.

Methods

Rectal afferent function was studied by recording CEPs in response to electrical rectal stimulation, expressed as sensory (ST) and pain thresholds (PT), amplitudes and latencies. Nineteen patients (16 females, mean age 41.3) with Rome 2-defined IBS were tested over 3 separate days. Electrical stimulation was facilitated by a bipolar ring electrode inserted 10 cm above the anus. CEP testing featured 4 cycles of 50 electrical stimulations, with the CEPs being averaged after every cycle. This testing paradigm has been previously shown to be the optimal protocol for maximising the signal-to-noise ratio. Intensity of the electrical stimulation was consistent with a subjective sensation of slight discomfort. Rectal electrical stimulation generated CEPs with distinct morphology, comprising P1, P2, P3, P4 and P5 peaks. Our previous research has defined the control reference values for P1 latency as a mean of 73.36 ms with a standard deviation of 18.54 ms. Therefore P1 latencies above 110 ms (2 SDs above the reference mean P1 latency) were considered abnormal. Hypersensitivity to rectal stimulation was defined as PT < 50mA. Psychological profiling included the Spielberger State-Trait Anxiety Inventory (SSTAI), an instrument powered to differentiate between one's anxiety levels at the present time from one's general tendency towards anxiety.

Results

Twelve IBS patients had normal P1 latencies and PTs. Four IBS patients had normal P1 latencies with decreased PTs, displaying visceral hypersensitivity. Two IBS patients had attenuated P1 latencies with increased PTs, exhibiting visceral hyposensitivity. One IBS patient had an increased P1 latency with decreased PT, suggesting hypervigilance. Mean SSTAI-State scores over 3 days were 40, 38.05 and 37.79; Mean SSTAI-Trait scores were 44.15, 41.1 and 41.88. Scores over 35 in either test were considered to be pathological.

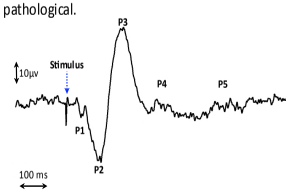
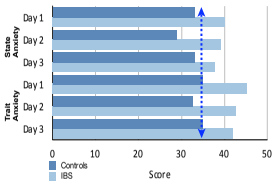


Figure 1: A cortical evoked potential produced by an electrical rectal stimulus. Morphology of the CEP is described in terms of P1, P2, P3, P4 and P5 waves.

Graph 1: Spielberger State and Trait Anxiety Scores in Controls and IBS patients over 3 days of testing



Graph 1: The Spielberger State Trait Anxiety Index is an instrument which is powered to differentiate one's present (state) anxiety from one's general levels of anxiety (trait). A value over 35 in each sub-scale identifies a case. IBS patients were significantly more anxious than controls for all of the days testing (with the exception of the Day 3 state anxiety which did not reach statistical significance).

Figure 2: A comparison of the cortical evoked potentials and anxiety scores in a healthy volunteer and two IBS patients over two days of testing

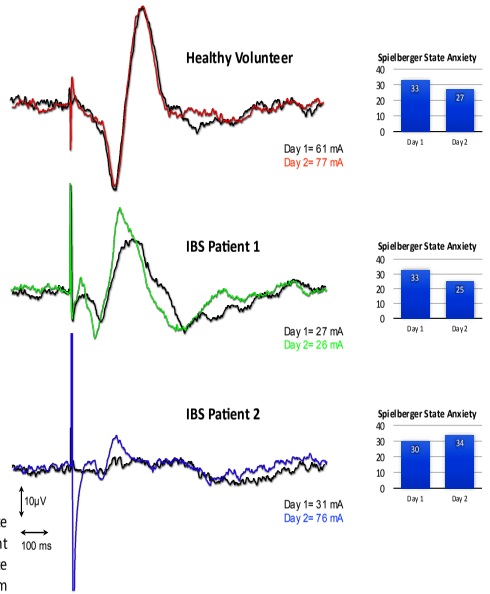


Figure 2: This shows the cortical evoked potentials for three different subjects (1 Control, 2 IBS) over 2 days of testing. The CEPs are accompanied by the testing thresholds (i.e. how much stimulus was administered to each patient) and the associated state anxiety figures (from the Spielberger State Trait Anxiety Index). All CEPs are drawn using the same scale.

The **Control** produces a robust, reproducible cortical potential using a stimulus of 61mA and then 77mA on Days 1 and 2 respectively. With increasing familiarity of the procedure, the state anxiety is diminished on Day 2.

IBS Patient 1 produces a marked cortical response with a stimulus intensity of just 27mA on Day 1 and 26mA on Day 2. This suggests that primary rectal afferent hypersensitivity is the driving force for his/her symptoms. Dropping anxiety levels on Day 2 do not alter the sensory and pain thresholds (which are calculated to produce the testing threshold).

IBS Patient 2 has an attenuated and indistinct CEP morphology on the first day of testing. On Day 2, an increased stimulus intensity is applied, and the CEP starts to adopt a more typical morphology. Anxiety levels are maintained over the 2 days. This suggests that a centrally-driven anxiety profile is the dominant factor in the development of the clinical phenotype.

Figure 3: Neurophysiological patient stratification

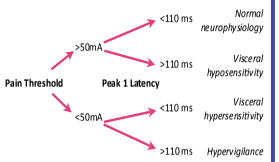


Figure 3: By using 110 ms as the cut-off point for Peak 1 latency (2 SDs above the control mean) and 50 mA as the cut-off for the testing threshold, patients can be grouped according to the putative aetiopathological mechanism.

Conclusion: Neurophysiological assessment of IBS patients using cortical evoked potentials exposes distinct clinical subgroups. The contribution of primary rectal hypersensitivity in the pathogenesis of pain appears to be limited. This study endorses the concept that IBS is a heterogeneous syndrome, and that both local and central mechanisms contribute to the developing clinical phenotype. Defining the contribution of each is crucial to allow effective targeting of drug therapy to the predominant underlying pathogenic mechanism.

Acknowledgements: This work was funded by a grant from GlaxoSmithKline R&D Ltd

Neurophysiological assessment of rectal afferent sensitivity in patients with Irritable Bowel Syndrome defines distinct clinical phenotypes

Objective assessment of rectal afferent sensitivity and primary cortical processing in patients with Irritable Bowel Syndrome

D. C. Bullas*, A. R. Hobson**, S. Gurmany*, K. J. Hicks**, G. E. Dukes**, M. A. Kamm*, W. S. Atkin*, N. Arebi*

* Physiology Department, St. Mark's Hospital Campus, Imperial College, Harrow, Middlesex, United Kingdom.

** Immuno-Inflammation CEDD, GlaxoSmithKline Medicines Research Centre, Stevenage, Hertfordshire, United Kingdom.

Background

Abnormalities in endogenous pain modulation and sensitisation of afferent nerve pathways may contribute to the development and maintenance of symptoms in Irritable Bowel Syndrome (IBS). Differentiating these distinct pathophysiological mechanisms in IBS is limited using conventional physiological assessments. Cortical evoked potentials (CEPs) allow a dynamic and objective assessment of visceral sensory function and could provide novel insight into IBS aetiology.

Aims and Methods

To measure primary rectal hypersensitivity by applying CEPs in IBS. Rome 2-positive patients and controls were recruited in a prospective study for 3 days of testing. Sensory (ST) and pain thresholds (PT) were calculated, and CEPs were captured at an stimulus intensity of 75% of the pain threshold (see below). CEP morphology includes P1, P2, P3, P4 and P5 components, distinguished by latency (ms) and amplitude (μV). Anxiety scores were provided by Spielberger State Trait Anxiety Index (SSTAI). Data were analysed using 1-way Anova, presented as mean differences ± 95% CI.

The methodology used to define the test intensity for electrical rectal stimulation required an assessment of sensory and pain thresholds before the evoked potentials could be captured. After placement of the electrical catheter, an intermittent electrical stimulus was administered with increasing stimulus intensity. Starting at 0mA and with a progressively increasing current, the patient would eventually record their first sensation of the stimulus, designated the sensory threshold. The stimulus intensity would continue to increase until a painful response was reported at which point the stimulus was discontinued. This was designated the pain threshold. This cycle is repeated two more times, allowing the average sensory and pain thresholds to be calculated. The test stimulus intensity is three quarters of the difference between the pain and sensory thresholds, subtracted from the pain threshold. In real terms this is equivalent to a subjective sensation of a slight discomfort. All the CEPs are now tested using this test stimulus intensity. Each stimulus was delivered at a frequency of 0.3 Hz using a 500 μsec duration square wave pulse.

Cortical evoked potentials were recorded according to the 10-20 international system of electroencephalogram recording. Electroencephalography recording electrodes were placed at the vertex, the right earlobe (reference electrode) and the neck (ground electrode). CEPs demonstrated characteristic morphology with positive and negative deflections from the baseline- P1, P2, P3, P4 and P5 peaks (see Figure 1).

Test stimulus intensity= ((Pain threshold - Sensory threshold) x 0.75) + Sensory threshold

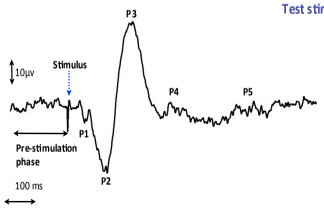


Figure 1: A cortical evoked potential induced by an electrical rectal stimulus. The pre-stimulation phase shows background cortical activity. The rectal stimulus is applied at 0 seconds. There is a brief time delay before a cortical response is seen, reflecting rectal afferent transmission. The individual components of the evoked potential have been assigned the terms P1, P2, P3, P4, P5 i.e. first peak, second peak etc.



Picture 1: The bipolar ring electrode (next to a ballpoint pen for reference). The catheter has a low profile and a highly flexible construction, making it more comfortable for the patient. It is inserted 10 cm into the rectum, secured into position, and then attached to the stimulus generator.



Pictures 2 & 3: A healthy volunteer demonstrating placement of the three recording electrodes according to the International 10-20 Electrode Placement System. The skin is gently prepared prior to electrode placement with a mild skin preparation lotion. Electrode conductance gel is then applied to interface between each electrode and the underlying skin.

Acknowledgements: This work was funded by a grant from GlaxoSmithKline R&D Ltd.

Results

33 IBS patients (27 females, mean age 40.1) and 15 controls (9 females, mean age 28.6) enrolled.

Latency of the evoked potentials (ms)

IBS subjects had increased P1 and P3 latencies on day 1 (20.89 (6.20,35.59)) and day 3 (30.64 (2.82,58.46)) respectively.

Amplitude of the evoked potentials (μV)

IBS subjects had decreased P2 amplitudes: (day 1: -6.96 (-13.89,-0.03)); (day 2: -8.62 (-16.11,-1.12)); (day 3: -10.26 (-16.96, -3.56)).
IBS subjects had decreased P3 amplitudes: (day 1: -8.46 (-14.21,-2.71)); (day 2: -3.36 (-5.66,-1.06)); (day 3: -10.65 (-16.13,-5.18)).
IBS subjects had decreased P4 amplitudes on day 1 (-3.36 (-5.66,-1.06)).

Sensory and pain thresholds (mA)

ST and PT did not differ between groups on day 1.
IBS subjects had increased sensory thresholds on day 2 of testing: (7.04 (1.21, 12.88)).
IBS subjects had decreased pain thresholds on day 3 of testing: (-15.63 (-29.24,-2.03)).

Spielberger Anxiety Scores

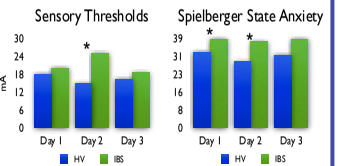
IBS subjects had increased state anxiety scores: SSTAI-State (day 1: 5.92 (0.43,11.40)); (day 2: 8.87 (2.58,15.17)).
IBS subjects had increased trait anxiety scores: SSTAI-Trait (day 1: 9.55 (3.45,15.65)); (day 2: 8.84 (2.35,15.32)); (day 3: 11.88 (5.02,18.74)).

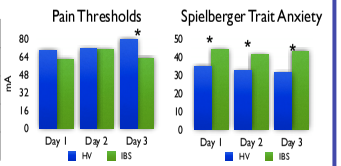
Table 1: Comparison of IBS patients against controls assessing the mean latency of the individual components of the rectal evoked potential over 3 days of testing

	Day 1 (m/s)	Day 1 CI (95%)	Day 2 (m/s)	Day 2 CI (95%)	Day 3 (m/s)	Day 3 CI (95%)
Peak 1 Latency	**20.89**	**(6.20, 35.59)**	18.24	(-0.43, 36.91)	35.55	(-20.13, 91.22)
Peak 2 Latency	17.63	(-1.96, 37.22)	19.37	(-3.42, 42.16)	19.17	(-4.07, 42.41)
Peak 3 Latency	15.64	(-11.01, 42.29)	19.85	(-2.52, 42.21)	**30.64**	**(2.82, 58.46)**
Peak 4 Latency	6.72	(-42.33, 55.76)	7.57	(-26.50, 41.63)	14.21	(-11.21, 39.63)
Peak 5 Latency	26.90	(-45.32, 99.12)	6.40	(-64.63, 77.43)	-8.76	(-70.56, 53.03)

Table 2: Comparison of IBS patients against controls assessing the mean amplitude of the individual components of the rectal evoked potential over 3 days of testing

	Day 1 (μV)	Day 1 CI (95%)	Day 2 (μV)	Day 2 CI (μV)	Day 3 (μV)	Day 3 CI (95%)
Peak 1 Amplitude	-1.85	(-4.84, 1.14)	-2.17	(-6.05, 1.70)	-2.09	(-5.12, 0.95)
Peak 2 Amplitude	**-6.96**	**(-13.89, -0.03)**	**-8.62**	**(-16.11, -1.12)**	**-10.26**	**(-16.96, -3.56)**
Peak 3 Amplitude	**-8.46**	**(-14.21, -2.71)**	**-9.69**	**(-15.60, -3.77)**	**-10.65**	**(-16.13, -5.18)**
Peak 4 Amplitude	**-3.36**	**(-5.66, -1.06)**	-1.94	(-4.49, 0.61)	-1.69	(-4.54, 1.16)

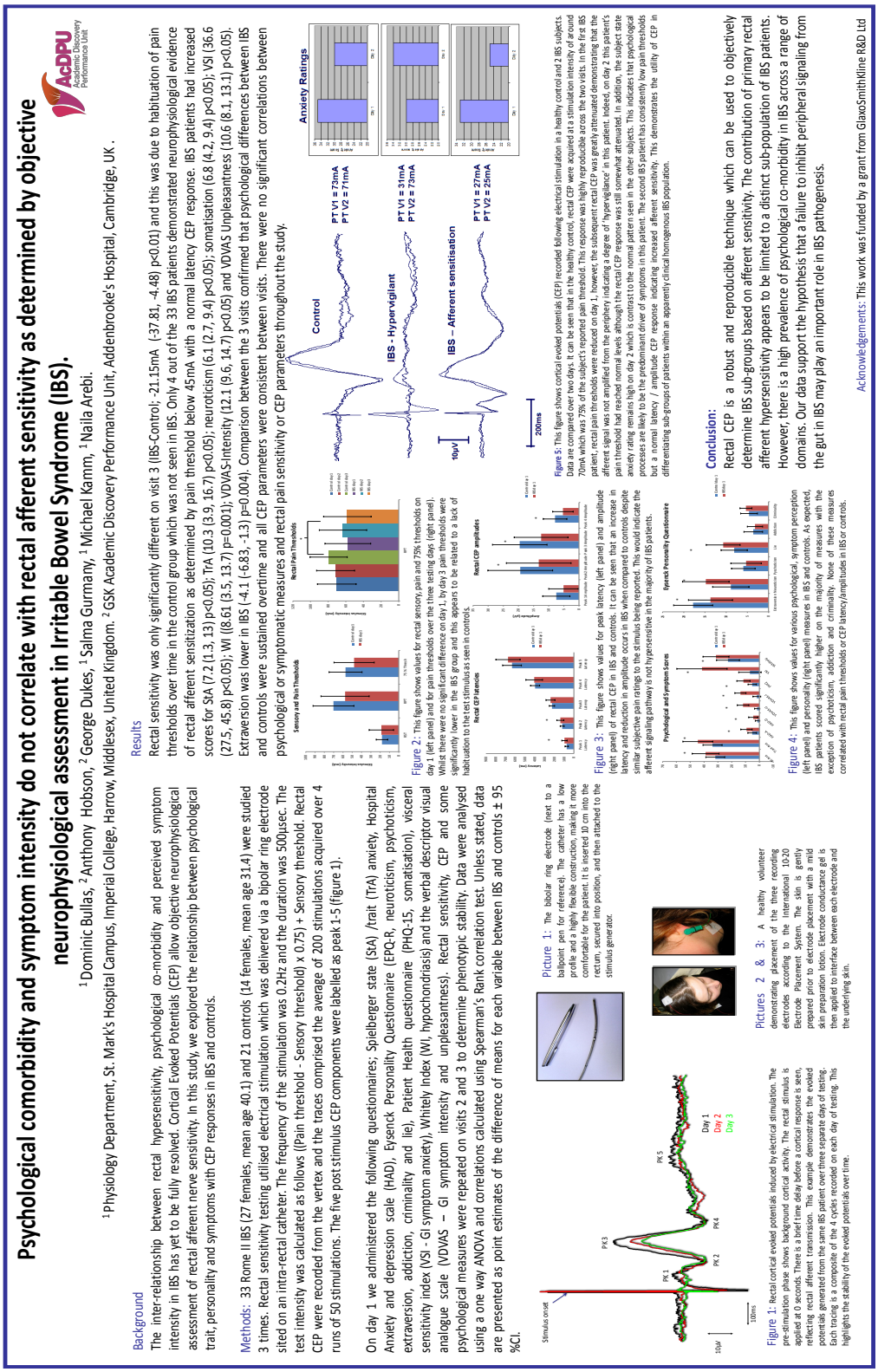




Conclusion

Analysis of cortical evoked potentials allows neurophysiological differentiation between IBS and controls at a group level. Primary rectal afferent hypersensitivity was not commonly seen in the IBS group. Pain thresholds showed a negative trend in IBS patients compared to controls on Days 1 & 2 that reached statistical significance on Day 3. This was a result of an increase in the pain thresholds of the controls rather than a relative decrease in IBS patients. In the context of persistent anxiety levels, this suggests that IBS patients maintain a level of visceral hypervigilance not shared by controls, who become acclimatised to the test protocol by Day 3. The inability to inhibit central pain amplification, in a process of habituation to repetitive noxious stimuli, may be important factor in the maintenance of IBS symptomatology.

Psychological comorbidity and symptom intensity do not correlate with rectal afferent sensitivity as determined by objective neurophysiological assessment in IBS



Psychological comorbidity and symptom intensity do not correlate with rectal afferent sensitivity as determined by objective neurophysiological assessment in Irritable Bowel Syndrome (IBS).

[1]Dominic Bullas, [2]Anthony Hobson, [2] George Dukes, [1] Salma Gurmany, [1] Michael Kamm, [1] Naila Arebi.

[1]Physiology Department, St. Mark's Hospital Campus, Imperial College, Harrow, Middlesex, United Kingdom. [2] GSK Academic Discovery Performance Unit, Addenbrooke's Hospital, Cambridge, UK .

Background

The inter-relationship between rectal hypersensitivity, psychological co-morbidity and perceived symptom intensity in IBS has yet to be fully resolved. Cortical Evoked Potentials (CEP) allow objective neurophysiological assessment of rectal afferent nerve sensitivity. In this study, we explored the relationship between psychological trait, personality and symptoms with CEP responses in IBS and controls.

Methods:

33 Rome II IBS (27 females, mean age 40.1) and 21 controls (14 females, mean age 31.4) were studied 3 times. Rectal sensitivity testing utilised electrical stimulation which was delivered via a bipolar ring electrode sited on an intra-rectal catheter. The frequency of the stimulation was 0.2Hz and the duration was 500μsec. The test intensity was calculated as follows ((Pain threshold - Sensory threshold) x 0.75) + Sensory threshold. Rectal CEP were recorded from the vertex and the traces comprised the average of 200 stimulations acquired over 4 runs of 50 stimulations. The five post stimulus CEP components were labelled as peak 1-5 (figure 1).

On day 1 we administered the following questionnaires; Spielberger state (StA) /trait (TrA) anxiety, Hospital Anxiety and depression scale (HAD), Eysenck Personality Questionnaire (EPQ-R, neuroticism, psychoticism, extraversion, addiction, criminality and lie), Patient Health questionnaire (PHQ-15, somatisation), visceral sensitivity index (VSI - GI symptom anxiety), Whitely Index (WI, hypochondriasis) and the verbal descriptor visual analogue scale (VDVAS – GI symptom intensity and unpleasantness). Rectal sensitivity, CEP and some psychological measures were repeated on visits 2 and 3 to determine phenotypic stability. Data were analysed using a one way ANOVA and correlations calculated using Spearman's Rank correlation test. Unless stated, data are presented as point estimates of the difference of means for each variable between IBS and controls ± 95 %CI.

Figure 1: Rectal cortical evoked potentials induced by electrical stimulation. The pre-stimulation phase shows background cortical activity. The rectal stimulus is applied at 0 seconds. There is a brief time delay before a cortical response is seen, reflecting rectal afferent transmission. This example demonstrates the evoked potentials generated from the same IBS patient over three separate days of testing. Each tracing is a composite of the 4 cycles recorded on each day of testing. This highlights the stability of the evoked potentials over time.

Picture 1: The bibolar ring electrode (next to a ballpoint pen for reference). The catheter has a low profile and a highly flexible construction, making it more comfortable for the patient. It is inserted 10 cm into the rectum, secured into position, and then attached to the stimulus generator.

Pictures 2 & 3: A healthy volunteer demonstrating placement of the three recording electrodes according to the International 10-20 Electrode Placement System. The skin is gently prepared prior to electrode placement with a mild skin preparation lotion. Electrode conductance gel is then applied to interface between each electrode and the underlying skin.

Results

Rectal sensitivity was only significantly different on visit 3 (IBS-Control: -21.15mA (-37.81, -4.48) p<0.01) and this was due to habituation of pain thresholds over time in the control group which was not seen in IBS. Only 4 out of the 33 IBS patients demonstrated neurophysiological evidence of rectal afferent sensitization as determined by pain threshold below 45mA with a normal latency CEP response. IBS patients had increased scores for StA (7.2 (1.3, 13) p<0.05); TrA (10.3 (3.9, 16.7) p<0.05); neuroticism (6.1 (2.7, 9.4) p<0.05); somatisation (6.8 (4.2, 9.4) p<0.05); VSI (36.6 (27.5, 45.8) p<0.05); WI ((8.61 (3.5, 13.7) p=0.001); VDVAS-Intensity (12.1 (9.6, 14.7) p<0.05) and VDVAS Unpleasantness ((10.6 (8.1, 13.1) p<0.05). Extraversion was lower in IBS (-4.1 (-6.83, -1.3) p=0.004). Comparison between the 3 visits confirmed that psychological differences between IBS and controls were sustained overtime and all CEP parameters were consistent between visits. There were no significant correlations between psychological or symptomatic measures and rectal pain sensitivity or CEP parameters throughout the study.

Figure 2: This figure shows values for rectal sensory, pain and 75% thresholds on day 1 (left panel) and for pain thresholds over the three testing days (right panel). Whilst there were no significant difference on day 1, by day 3 pain thresholds were significantly lower in the IBS group and this appears to be related to a lack of habituation to the test stimulus as seen in controls.

Figure 3: This figure shows values for peak latency (left panel) and amplitude (right panel) of rectal CEP in IBS and controls. It can be seen that an increase in latency and reduction in amplitude occurs in IBS when compared to controls despite similar subjective pain ratings to the stimulus being reported. This would indicate the afferent signalling pathways is not hypersensitive in the majority of IBS patients.

Figure 4: This figure shows values for various psychological, symptom perception (left panel) and personality (right panel) measures in IBS and controls. As expected, IBS patients scored significantly higher on the majority of measures with the exception of psychoticism, addiction and criminality. None of these measures correlated with rectal pain thresholds or CEP latency/amplitudes in IBS or controls.

Figure 5: This figure shows cortical evoked potentials (CEP) recorded following electrical stimulation in a healthy control and 2 IBS subjects. Data are compared over two days. It can be seen that in the healthy control, rectal CEP were acquired at a stimulation intensity of around 70mA which was 75% of the subject's reported pain threshold. This response was highly reproducible across the two visits. In the first IBS patient, rectal pain thresholds were reduced on day 1, however, the subsequent rectal CEP was greatly attenuated demonstrating that the afferent signal was not amplified from the periphery indicating a degree of 'hypervigilance' in this patient. Indeed, on day 2 this patient's pain threshold had reached normal levels although the rectal CEP response was still somewhat attenuated. In addition, the subject state anxiety rating remains high on day 2 which is contrast to the normal pattern seen in the other subjects. This indicates that psychological processes are likely to be the predominant driver of symptoms in this patient. The second IBS patient has consistently low pain thresholds but a normal latency / amplitude CEP response indicating increased afferent sensitivity. This demonstrates the utility of CEP in differentiating sub-groups of patients within an apparently clinical homogenous IBS population.

Conclusion:

Rectal CEP is a robust and reproducible technique which can be used to objectively determine IBS sub-groups based on afferent sensitivity. The contribution of primary rectal afferent hypersensitivity appears to be limited to a distinct sub-population of IBS patients. However, there is a high prevalence of psychological co-morbidity in IBS across a range of domains. Our data support the hypothesis that a failure to inhibit peripheral signaling from the gut in IBS may play an important role in IBS pathogenesis.

Acknowledgements: This work was funded by a grant from GlaxoSmithKline R&D Ltd

UEGW/WCOG GASTRO 2009 POSTER, 23.11.09, LONDON

High depression and anxiety scores in a systematic review of Psychological Questionnaires in Irritable Bowel Syndrome

C. Elliott, M. A. Kamm, D. Bullas, S. R. Gurmany, N. Arebi

INTRODUCTION

Irritable bowel syndrome (IBS) is associated with psychological co-morbidity (PCM). Specific psychological questionnaires (PQ) have been used to define PCM prevalence and applied in therapeutic trials. Numerous questionnaires are available with emphasis on different psychological domains. PCM may indicate the need for psychological therapy

AIMS & METHODS

1) to determine types and frequency of PQ used to record PCM in IBS 2) to compare score differences between IBS and controls. A PubMed literature search (1980 to 2008) used search terms questionnaire/scores with a list of psychological conditions and IBS. Excluded studies: population with primary psychological or psychiatric disorder, IBS treatment studies or quality of life or well-being PQ. For aim 2 only studies that reported controls scores were assessed. Results are expressed as mean scores and 95% confidence intervals (CI).

RESULTS

From a total 371 studies, 68 studies met the inclusion criteria. Fifty-three studies were analysed. A total of 46 different PQ were identified. The number of studies using each PQ is shown in parenthesis: Scl-90(14), Hospital Anxiety and Depression Scale (HADS)(10), Eysenck Personality Inventory (EPI)(8), Diagnostic Interview Schedule (DIS- DSM-III-R)(8), Minnesota Multiphasic Personality Inventory (MMPI) (8), Short Form (SF-36)(7), Beck Depression Inventory (BDI)(7) Hamilton Depression Scale (HDS) (6) Spielberger State/trait Anxiety (SSTAI) (5). The PQ in remaining 36 studies was infrequently used (1-3). Mean score differences between controls and IBS were greatest for depression (7.0) and anxiety (8.3)irrespective of PQ type (table). DIS- DSM-III-R reported % of patients above a threshold. Scores for somatisation, obsessive compulsive, interpersonal sensitivity, phobic anxiety, paranoid ideation and personality by MMPI and EPI were only marginally greater for IBS compared to controls.

TABLE 1:

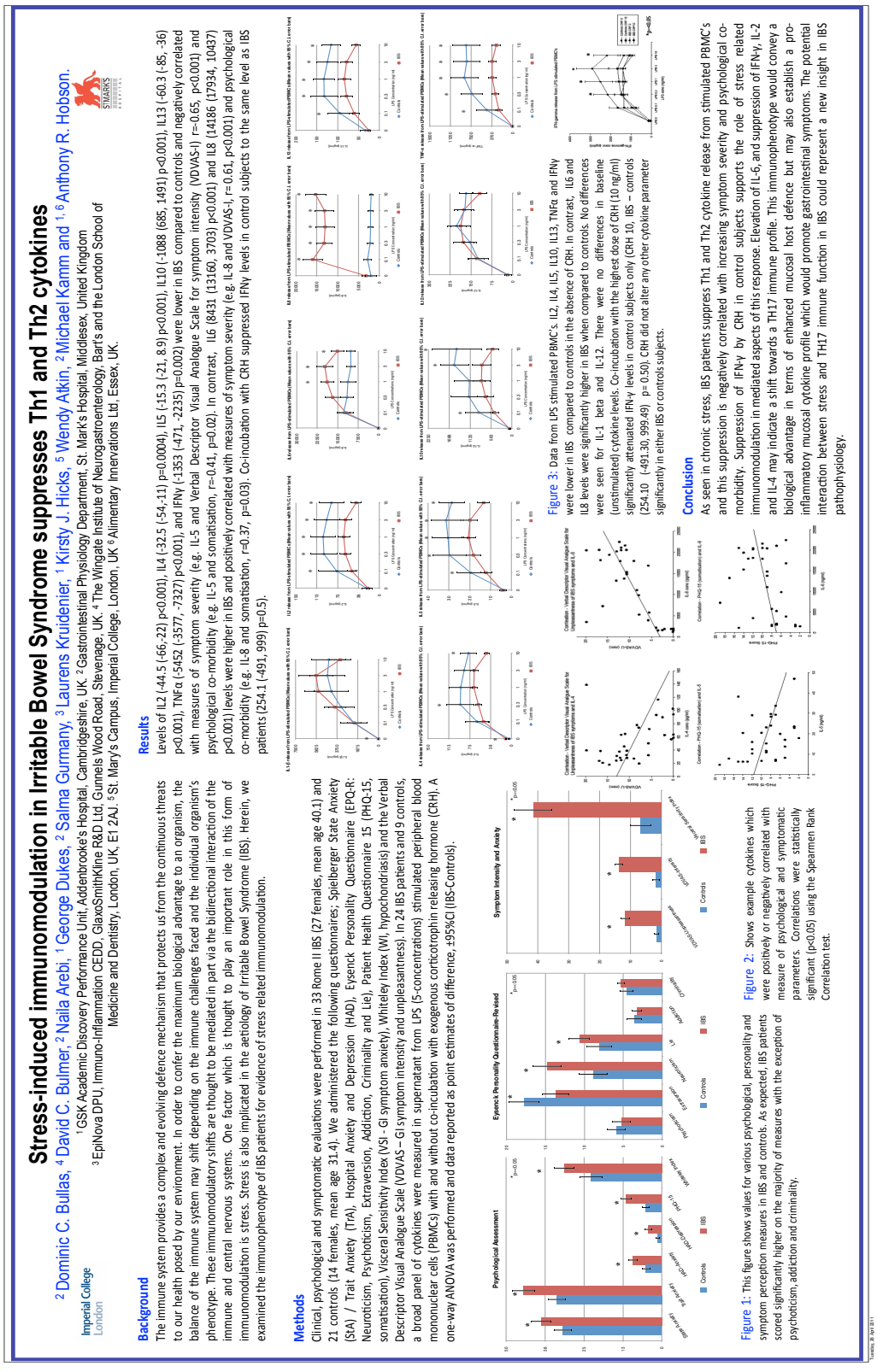
Depression and anxiety scores compared to controls

†	mean IBS score (95% CI)	IBS studies n	mean control score (95% CI)	control studies n
BDI	11.3 (9-13.6)	7	3.5(2.2-5.2)	5
HDS	10.1(7,1-13.1)	5	1.7(0.1-3.3)	2
HADS(depression)	5.6(3.7-7.5)	6	1.6(0.5-2.7)	3
HADS(anxiety)	7.8(6-9.6)	8	3.4(2.8-4)	3
SSAI	47.4(42.5-52.9)	3	35.5(33.9-37.1)	1
STAI	47.2(45.8-48.6)	4	41.2(41.1-41.3)	2
SCL-90 Depression	58.2 (56 ñ 60.3)	5	49.2 (47.5 ñ 50.9)	2
SCL-90 Anxiety	54.3(52 ñ 56.6)	5	47.5 (32.5 ñ 52.5),	2

CONCLUSION

46 PQ have been used to describe IBS PCM. Control PQ scores are marginally lower than IBS patients for all domains except for depression and anxiety. These indicate that whilst PCM is common, depression and anxiety are the more marked manifestations of IBS. Psychological assessment in clinical practice is should focus on eliciting these 2 conditions.

Stress-induced immunomodulation in Irritable Bowel Syndrome suppresses TH1 and TH2 cytokines



Stress-induced immunomodulation in Irritable Bowel Syndrome suppresses Th1 and Th2 cytokines

[2] Dominic C. Bullas, [4] David C. Bulmer, [2] Naila Arebi, [1] George Dukes, [2] Salma Gurmany, [3] Laurens Kruidenier, [1] Kirsty J. Hicks, [5] Wendy Atkin, [2] Michael Kamm and [1,6] Anthony R. Hobson.

Imperial College London

[1] GSK Academic Discovery Performance Unit, Addenbrooke's Hospital, Cambridgeshire, UK. [2] Gastrointestinal Physiology Department, St. Mark's Hospital, Middlesex, United Kingdom
[3] EpiNova DPU, Immuno-Inflammation CEDD, GlaxoSmithKline R&D Ltd, Gunnels Wood Road, Stevenage, UK. [4] The Wingate Institute of Neurogastroenterology, Bart's and the London School of Medicine and Dentistry, London, UK, E1 2AJ. [5] St. Mary's Campus, Imperial College, London, UK [6] Alimentary Innervations Ltd, Essex, UK.

Background

The immune system provides a complex and evolving defence mechanism that protects us from the continuous threats to our health posed by our environment. In order to confer the maximum biological advantage to an organism, the balance of the immune system may shift depending on the immune challenges faced and the individual organism's phenotype. These immunomodulatory shifts are thought to be mediated in part via the bidirectional interaction of the immune and central nervous systems. One factor which is thought to play an important role in this form of immunomodulation is stress. Stress is also implicated in the aetiology of Irritable Bowel Syndrome (IBS). Herein, we examined the immunophenotype of IBS patients for evidence of stress related immunomodulation.

Methods

Clinical, psychological and symptomatic evaluations were performed in 33 Rome II IBS (27 females, mean age 40.1) and 21 controls (14 females, mean age 31.4). We administered the following questionnaires; Spielberger State Anxiety (StA) / Trait Anxiety (TrA), Hospital Anxiety and Depression (HAD), Eysenck Personality Questionnaire (EPQ-R: Neuroticism, Psychoticism, Extraversion, Addiction, Criminality and Lie), Patient Health Questionnaire 15 (PHQ-15, somatisation), Visceral Sensitivity Index (VSI - GI symptom anxiety), Whiteley Index (WI, hypochondriasis) and the Verbal Descriptor Visual Analogue Scale (VDVAS – GI symptom intensity and unpleasantness). In 24 IBS patients and 9 controls, a broad panel of cytokines were measured in supernatant from LPS (5-concentrations) stimulated peripheral blood mononuclear cells (PBMCs) with and without co-incubation with exogenous corticotrophin releasing hormone (CRH). A one-way ANOVA was performed and data reported as point estimates of difference, ±95%CI (IBS-Controls).

Results

Levels of IL2 (-44.5 (-66,-22) p<0.0001), IL4 (-32.5 (-54,-11) p=0.0004), IL5 (-15.3 (-21, 8.9) p<0.001), IL10 (-1088 (685, 1491) p<0.001), IL13 (-60.3 (-85, -36) p<0.001), TNFα (-5452 (-3577, -7327) p<0.001), and IFNγ (-1353 (-471, -2235) p=0.002) were lower in IBS compared to controls and negatively correlated with measures of symptom severity (e.g. IL-5 and Verbal Descriptor Visual Analogue Scale for symptom intensity (VDVAS-I) r=-0.65, p<0.001) and psychological co-morbidity (e.g. IL-5 and somatisation, r=-0.41, p=0.02). In contrast, IL6 (8431 (13160, 3703) p<0.001) and IL8 (14186 (17934, 10437) p<0.001) levels were higher in IBS and positively correlated with measures of symptom severity (e.g. IL-8 and VDVAS-I, r= 0.61, p<0.001) and psychological co-morbidity (e.g. IL-8 and somatisation, r=0.37, p=0.03). Co-incubation with CRH suppressed IFNγ levels in control subjects to the same level as IBS patients (254.1 (-491, 999) p=0.5).

Figure 3: Data from LPS stimulated PBMC's. IL2, IL4, IL5, IL10, IL13, TNFα and IFNγ were lower in IBS compared to controls in the absence of CRH. In contrast, IL6 and IL8 levels were significantly higher in IBS when compared to controls. No differences were seen for IL-1 beta and IL-12. There were no differences in baseline (unstimulated) cytokine levels. Co-incubation with the highest dose of CRH (10 ng/ml) significantly attenuated IFNγ levels in control subjects only (CRH 10, IBS – controls (254.10 (-491.30, 999.49) p= 0.50). CRH did not alter any other cytokine parameter significantly in either IBS or controls subjects.

Figure 1: This figure shows values for various psychological, personality and symptom perception measures in IBS and controls. As expected, IBS patients scored significantly higher on the majority of measures with the exception of psychoticism, addiction and criminality.

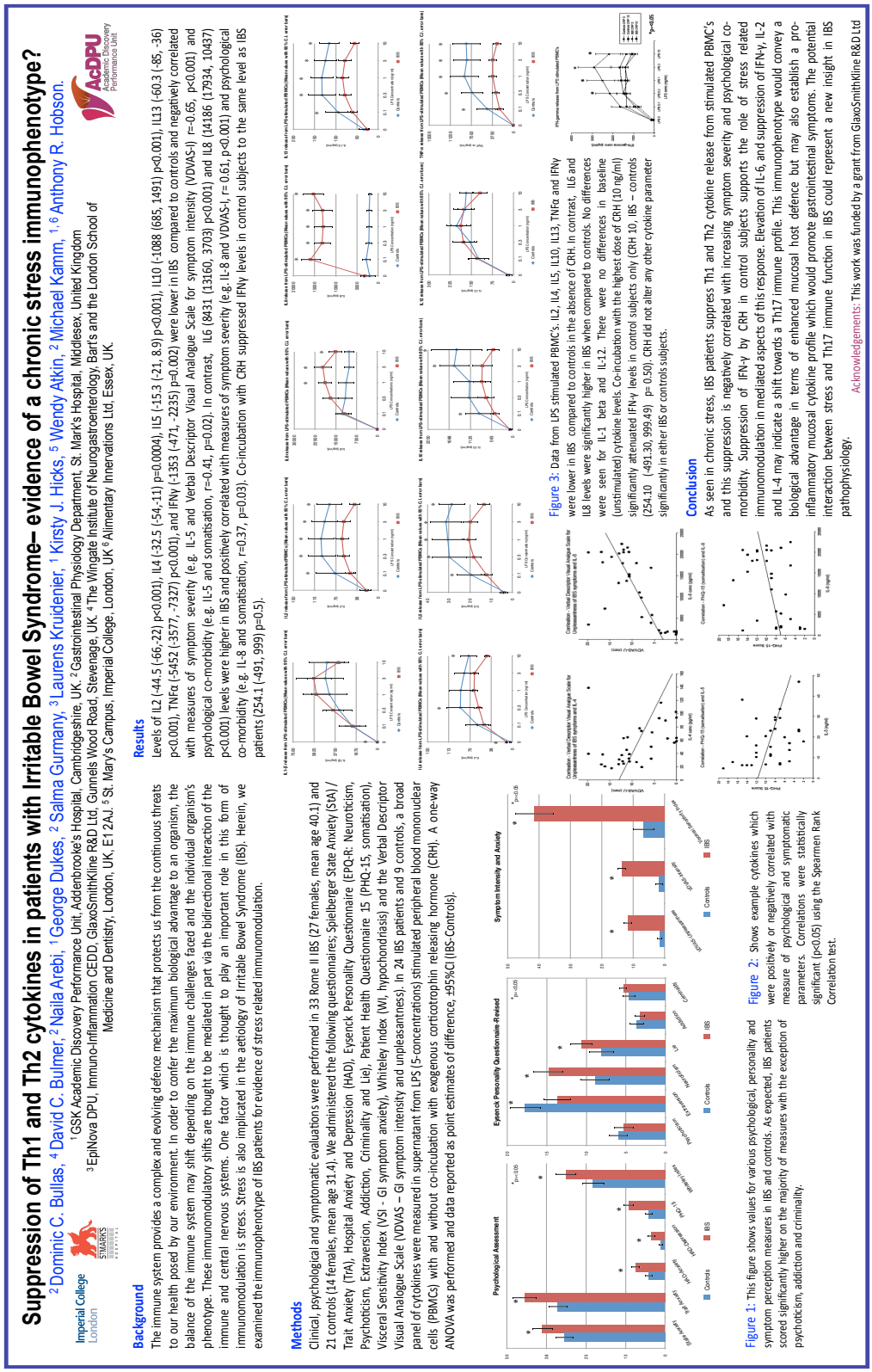
Figure 2: Shows example cytokines which were positively or negatively correlated with measure of psychological and symptomatic parameters. Correlations were statistically significant (p<0.05) using the Spearmen Rank Correlation test.

Conclusion

As seen in chronic stress, IBS patients suppress Th1 and Th2 cytokine release from stimulated PBMC's and this suppression is negatively correlated with increasing symptom severity and psychological co-morbidity. Suppression of IFNγ by CRH in control subjects supports the role of stress related immunomodulation in mediated aspects of this response. Elevation of IL-6, and suppression of IFNγ, IL-2 and IL-4 may indicate a shift towards a TH17 immune profile. This immunophenotype would convey a biological advantage in terms of enhanced mucosal host defence but may also establish a pro-inflammatory mucosal cytokine profile which would promote gastrointestinal symptoms. The potential interaction between stress and TH17 immune function in IBS could represent a new insight in IBS pathophysiology.

AGA INSTITUTE, DIGESTIVE DISEASE WEEK, NEW ORLEANS, LA, MAY 4TH 2010

Suppression of TH1 and TH2 cytokines in patients with Irritable Bowel Syndrome- evidence of a chronic stress immunophenotype?



Suppression of Th1 and Th2 cytokines in patients with Irritable Bowel Syndrome– evidence of a chronic stress immunophenotype?

[2]Dominic C. Bullas, [4]David C. Bulmer, [2]Naila Arebi, [1]George Dukes, [2]Salma Gurmany, [3]Laurens Kruidenier, [1]Kirsty J. Hicks, [5]Wendy Atkin, [2]Michael Kamm, [1,6]Anthony R. Hobson.

[1]GSK Academic Discovery Performance Unit, Addenbrooke's Hospital, Cambridgeshire, UK. [2]Gastrointestinal Physiology Department, St. Mark's Hospital, Middlesex, United Kingdom
[3]EpiNova DPU, Immuno-Inflammation CEDD, GlaxoSmithKline R&D Ltd, Gunnels Wood Road, Stevenage, UK. [4]The Wingate Institute of Neurogastroenterology, Bart's and the London School of Medicine and Dentistry, London, UK, E1 2AJ. [5]St. Mary's Campus, Imperial College, London, UK [6]Alimentary Innervations Ltd, Essex, UK.

Background

The immune system provides a complex and evolving defence mechanism that protects us from the continuous threats to our health posed by our environment. In order to confer the maximum biological advantage to an organism, the balance of the immune system may shift depending on the immune challenges faced and the individual organism's phenotype. These immunomodulatory shifts are thought to be mediated in part via the bidirectional interaction of the immune and central nervous systems. One factor which is thought to play an important role in this form of immunomodulation is stress. Stress is also implicated in the aetiology of Irritable Bowel Syndrome (IBS). Herein, we examined the immunophenotype of IBS patients for evidence of stress related immunomodulation.

Methods

Clinical, psychological and symptomatic evaluations were performed in 33 Rome II IBS (27 females, mean age 40.1) and 21 controls (14 females, mean age 31.4). We administered the following questionnaires; Spielberger State Anxiety (StA) / Trait Anxiety (TrA), Hospital Anxiety and Depression (HAD), Eysenck Personality Questionnaire (EPQ-R: Neuroticism, Psychoticism, Extraversion, Addiction, Criminality and Lie), Patient Health Questionnaire 15 (PHQ-15, somatisation), Visceral Sensitivity Index (VSI - GI symptom anxiety), Whiteley Index (WI, hypochondriasis) and the Verbal Descriptor Visual Analogue Scale (VDVAS – GI symptom intensity and unpleasantness). In 24 IBS patients and 9 controls, a broad panel of cytokines were measured in supernatant from LPS (5-concentrations) stimulated peripheral blood mononuclear cells (PBMCs) with and without co-incubation with exogenous corticotrophin releasing hormone (CRH). A one-way ANOVA was performed and data reported as point estimates of difference, ±95%CI (IBS-Controls).

Figure 1: This figure shows values for various psychological, personality and symptom perception measures in IBS and controls. As expected, IBS patients scored significantly higher on the majority of measures with the exception of psychoticism, addiction and criminality.

Figure 2: Shows example cytokines which were positively or negatively correlated with measure of psychological and symptomatic parameters. Correlations were statistically significant (p<0.05) using the Spearmen Rank Correlation test.

Results

Levels of IL2 (-44.5 (-66,-22) p<0.0001), IL4 (-32.5 (-54,-11) p=0.0004), IL5 (-15.3 (-21, 8.9) p<0.001), IL10 (-1088 (685, 1491) p<0.001), IL13 (-60.3 (-85, -36) p<0.001), TNFα (-5452 (-3577, -7327) p<0.001), and IFNγ (-1353 (-471, -2235) p=0.002) were lower in IBS compared to controls and negatively correlated with measures of symptom severity (e.g. IL-5 and Verbal Descriptor Visual Analogue Scale for symptom intensity (VDVAS-I) r=-0.65, p<0.001) and psychological co-morbidity (e.g. IL-5 and somatisation, r=-0.41, p=0.02). In contrast, IL6 (8431 (13160, 3703) p<0.001) and IL8 (14186 (17934, 10437) p<0.001) levels were higher in IBS and positively correlated with measures of symptom severity (e.g. IL-8 and VDVAS-I, r= 0.61, p<0.001) and psychological co-morbidity (e.g. IL-8 and somatisation, r=0.37, p=0.03). Co-incubation with CRH suppressed IFNγ levels in control subjects to the same level as IBS patients (254.1 (-491, 999) p=0.5).

Figure 3: Data from LPS stimulated PBMC's. IL2, Il4, IL5, IL10, IL13, TNFα and IFNγ were lower in IBS compared to controls in the absence of CRH. In contrast, IL6 and IL8 levels were significantly higher in IBS when compared to controls. No differences were seen for IL-1 beta and IL-12. There were no differences in baseline (unstimulated) cytokine levels. Co-incubation with the highest dose of CRH (10 ng/ml) significantly attenuated IFNγ levels in control subjects only (CRH 10, IBS – controls (254.10 (-491.30, 999.49) p= 0.50). CRH did not alter any other cytokine parameter significantly in either IBS or controls subjects.

Conclusion

As seen in chronic stress, IBS patients suppress Th1 and Th2 cytokine release from stimulated PBMC's and this suppression is negatively correlated with increasing symptom severity and psychological co-morbidity. Suppression of IFN-γ by CRH in control subjects supports the role of stress related immunomodulation in mediated aspects of this response. Elevation of IL-6, and suppression of IFN-γ, IL-2 and IL-4 may indicate a shift towards a Th17 immune profile. This immunophenotype would convey a biological advantage in terms of enhanced mucosal host defence but may also establish a pro-inflammatory mucosal cytokine profile which would promote gastrointestinal symptoms. The potential interaction between stress and Th17 immune function in IBS could represent a new insight in IBS pathophysiology.

Acknowledgements: This work was funded by a grant from GlaxoSmithKline R&D Ltd

Am J Physiol Gastrointest Liver Physiol 300: G1086–G1093, 2011.
First published February 24, 2011; doi:10.1152/ajpgi.00553.2010.

Distinct neurophysiological profiles in irritable bowel syndrome

Naila Arebi,[1]* Dominic C. Bullas,[1]* George E. Dukes,[2] Salma Gurmany,[1] Kirsty J. Hicks,[2] Michael A. Kamm,[4,5] and Anthony R. Hobson[2,3]

[1]*Gastrointestinal Physiology Department, St. Mark's Hospital Campus, Imperial College, Harrow, Middlesex,* [2]*GlaxoSmithKline Research and Development, Stevenage,* [3]*The Neurogastroenterology Diagnostic Centre, Princess Grace Hospital,* [5]*Imperial College, London, United Kingdom; and* [4]*St. Vincent's Hospital and University of Melbourne, Melbourne, Australia*

Submitted 20 December 2010; accepted in final form 23 February 2011

Arebi N, Bullas DC, Dukes GE, Gurmany S, Hicks KJ, Kamm MA, Hobson AR. Distinct neurophysiological profiles in irritable bowel syndrome. *Am J Physiol Gastrointest Liver Physiol* 300: G1086–G1093, 2011. First published February 24, 2011; doi:10.1152/ajpgi.00553.2010.—The objective of this study was to determine whether cortical evoked potentials (CEPs) can define neurophysiological patterns in irritable bowel syndrome (IBS). In this prospective study of consecutive patients attending secondary and tertiary centers, patients with Rome II-defined IBS underwent rectal sensory and pain threshold (RST and RPT, respectively) testing with electrical stimulation on three separate visits. CEPs were collated for 75% pain thresholds, and anxiety [Spielberger State-Trait Anxiety Inventory (SSTAI)] questionnaires were completed. Subjects were 33 IBS patients (27 female, mean age 40.1 yr) and 21 healthy controls (14 female, mean age 31.4 yr). At *visit 3,* RPT was significantly lower [mean (95% CI)] in IBS patients than in control subjects: 58.2 mA (48.0–68.5) vs. 79.5 mA (69.3–89.6) ($P < 0.01$). No significant differences were observed in CEP latencies and amplitudes between *visits 1, 2,* and *3* within each group, except P2 latency for controls ($P = 0.04$) and N2 latency ($P = 0.04$) and N2 amplitude ($P = 0.02$) for IBS patients. Group comparisons showed significant differences in 3-day mean RPT, CEP amplitudes, and CEP latencies between IBS patients and controls. RPT <50 mA and P1 latency >106 ms were identified four IBS subgroups: 24% were hypersensitive, 12% were hypervigilant, 15% were hyposensitive, and 49% exhibited normal P1 latency and pain threshold. CEPs are reliable and reproducible measures of early sensory processing. Identification of four IBS neurophysiological patterns highlights its heterogeneous nature. These findings mark the first step toward personalized medicine in IBS, whereby therapy may be directed at the underlying physiological process.

brain-gut interaction; visceral sensitivity; gastrointestinal physiology

ABDOMINAL PAIN IS THE CARDINAL symptom of irritable bowel syndrome (IBS), with associated diagnostic symptoms such as alterations in stool frequency or consistency (7). Visceral hypersensitivity is a recognized physiological process associated with pain in IBS (20). Several mechanisms may contribute to the low pain threshold observed in this condition. Peripheral sensitization of visceral afferent nerves activated by previous injury or infection, mediated via inflammatory mediators such as prostaglandins, is closely integrated with central sensitization of spinal afferents (21, 26). Once chronic sensitization is established, the two processes become indistinguishable, as they contribute to the development of visceral hypersensitivity in functional gut disorders (5, 21, 25, 26). More recently, perception of gut stimuli and postprandial symptoms in IBS were shown to be enhanced during stressful conditions (10, 22). As the treatment and management strategies deployed to ameliorate these different aberrant mechanisms are distinct (i.e., analgesics vs. psychological therapy), identifying the predominant underlying pathophysiology remains an important clinical objective.

The subjective end point of pain or discomfort when assessing visceral sensation remains inadequate because of susceptibility to response bias related to the psychological state, with a higher tendency of patients with functional gut disorders to report pain (4, 31). The lack of a single robust objective clinical marker to quantify visceral hypersensitivity has hindered progress in IBS drug development. Much of this difficulty lies in the complex multifaceted nature of the condition, compounded by the strong emotional and neurobehavioral components of pain.

Surrogate measurements of visceral pain can be obtained from visceral function studies, such as the barostat, or metabolic neuroimaging techniques, such as PET and functional MRI (1, 18). Evaluation of pain using these tools had several limitations, including anticipation, habituation, and lack of non-stimulus-specific activity (23, 33). Some of the limitations were overcome by magnetoencephalography (MEG) (15). However, as MEG is available in only a few centers worldwide, extrapolation of MEG data to EEG recording of cerebral evoked potentials (CEPs) was subsequently developed as a possible clinical tool with an objective evaluation of visceral pain. CEPs are measurements of electrical potentials, generated by cortical neurons following a series of repeated sensory stimuli, recorded using scalp surface electrodes. Stimulus-specific CEPs occur at a fixed time after each stimulus, while other brain activity does not; stimulus-specific CEPs may be "extracted" from the brain's background as a distinct waveform.

CEPs have been used to understand pain transmission in the noncardiac chest pain (NCCP) disease model, where CEPs represent objective neurophysiological correlates of afferent transmission and processing within the brain (27). With esophageal stimulation, as stimulation intensity and sensory perception increases toward pain, P1 latency decreases, and wave amplitude increases (14). This phenomenon reflects the recruitment of an increasing number of afferents. The relationship between amplitude, latency, and intensity of sensory perception favors visceral CEP as an objective neurophysiological correlate of gastrointestinal sensory processing (14). Subsequent studies with CEP have shown that a homogeneous NCCP group could be differentiated into three distinct neurophysiological phenotypes (16).

* N. Arebi and D. C. Bullas contributed equally to this work.
Address for reprint requests and other correspondence: N. Arebi, St. Mark's Hospital, Watford Rd., Harrow, London HA1 3UJ, UK (e-mail: naila.arebi@imperial.ac.uk).

0193-1857/11 Copyright © 2011 the American Physiological Society

Downloaded from ajpgi.physiology.org on September 13, 2011

The purpose of the present study was to determine whether distinct neurophysiological phenotypes occur in IBS. The primary aim was to test the reliability of CEPs in response to rectal electrical stimulation over time in IBS. Secondary aims were to correlate these profiles with sensory and psychological measures.

MATERIALS AND METHODS

Consecutive IBS patients were recruited from the outpatient clinics at a tertiary and a secondary care hospital. Patients were included if they fulfilled the Rome II criteria for IBS. Investigations were undertaken at the discretion of the attending physician. Exclusion criteria included unexplained weight loss, psychiatric disorder, acute infection, recent antibiotic treatment, antidepressant therapy, anxiolytics, and anti-inflammatory or pain medication. Healthy volunteers were recruited concomitantly as the control group by local advertisement. All participants gave informed consent. The study was approved by the Harrow Ethics Committee.

Protocol

Each participant underwent 3 nonconsecutive days of neurophysiological assessment within a 3-mo period. At each visit, subjects underwent rectal electrical stimulation to determine sensory and pain thresholds; CEPs to the rectal stimulus were subsequently recorded. Female patients were tested during the follicular phase of the menstrual cycle.

Assessment of Rectal Sensitivity

Rectal stimulation. The rectal catheter (Gaeltec, Dunvegan, Isle of Skye, Scotland, UK) featured two 2-mm bipolar ring electrodes sited 1 cm apart. The electrodes were positioned 10 cm proximal to the anal verge. The catheter was connected to a constant-current stimulator (model DS7A, Digitimer), which was controlled by a trigger generator (model DG2, Digitimer). The stimulus frequency was 0.5 Hz using 500-μs square-wave pulses. The intensity of the stimulus was manually adjusted between 0 and 100 mA.

Visual analog scale. Subjects graded the stimulus intensity using a visual analog scale (VAS) scored between 1 and 6 as follows: "unaware of the stimulus" was scored as 1, "slight sensation" as 2, "definite sensation" as 3, "slight discomfort" as 4 "uncomfortable" as 5, and "painful" as 6. This scale has been used previously in CEP studies and showed correlation between stimulation intensity and CEP responses (16).

Rectal sensory and pain thresholds. Rectal sensory and pain thresholds (RST and RPT, respectively) were determined by assessing response in 2-mA increments. The first electrical stimulus perception was defined as the sensory threshold, or VAS = 2. Further stimulus intensity increases were recorded via VAS until sensation was perceived as painful (defined as the pain threshold, or VAS = 6). This ascending electrical stimulation cycle was repeated twice to calculate average RST and RPT over three cycles. The stimulus intensity used to elicit CEP was equivalent to the RST plus 75% of the difference between the RST and RPT (75% RPT) and has been validated in previous studies (13).

Assessment of CEPs

Placement of cortical electrodes. Silver-silver chloride surface electrodes were placed on the vertex (active electrode), earlobe (reference electrode), and neck (ground electrode) according to the International 10-20 System of EEG electrode placement. The electrodes were fixed using electrode gel or paste (Elebix, Nihon Kohden) after the skin was scrubbed with a mild abrasive (Omniprep, Weaver & Aurora) to maintain scalp impedance at <5 kΩ.

CEP recordings. CEPs were recorded with subjects lying still and their gaze fixed on a stationary object to minimize motor artifacts. The rectal stimulus was delivered at the calculated testing intensity over four cycles of 50 electrical stimulations spaced at 5-s intervals. A 5-min pause interspersed each cycle. Each cycle of 50 stimulations was averaged to enhance signal clarity. The patient was given 5 min to relax after each cycle before testing resumed. At the conclusion of each day of testing, four averaged CEP waveforms were calculated.

CEP acquisition and analysis. Data were acquired using a programmable signal conditioner (model 1902, Cambridge Electronics Design), as previously described (13). Rectal CEPs were analyzed according to morphology, latency, and amplitude of the waveforms. CEPs were averaged after each cycle and then compared with other cycles on the same day of testing (intraday analysis) and on other days of testing (interday analysis). Four main CEP components were recognized in previous studies.

Definition of terms. The main CEP peak components are P1, N1, P2, and N2. Neurophysiological convention describes negative potentials as upward deflections (N1 and N2) and positive potentials as downward deflections (P1 and P2). Latency refers to time (in ms) from the stimulus trigger to the peak component. Amplitude is defined as the voltage difference between consecutive CEP peaks (Fig. 1).

Early recorded CEP complex represents stimulus-specific activation of the primary or secondary somatosensory cortex and insula and depends on transmitted afferent signal strength intensity. Cortical activity of ~250 ms or more after stimulation reflects brain endogenous processing of the afferent information and represents a more general "arousal"-type response. The relationship between CEP morphology and pain thresholds was examined in a previous study (16) and defined as follows. *1*) Afferent hypersensitivity refers to pain perception at relatively low stimulus intensity with a normal-latency P1 component. *2*) Hypervigilance refers to pain perception at low stimulation intensity, but with prolonged-latency P1 responses (indicating that afferent signaling pathways are not sensitized, instead patients were "amplifying" normal-intensity stimuli once the stimulus reached the brain). *3*) Hyposensitivity refers to maximum stimulus application (limited by the stimulator and safety reasons) without eliciting a normal CEP response.

Withdrawals. All participants completed the first day of CEP testing (*visit 1*). At *visit 2*, two IBS patients and two controls dropped out for the following reasons: failure to attend, technical failure of electronic CEP data capture, pregnancy, and difficulty in attending hospital. At *visit 3*, a further two IBS patients withdrew: one failed to attend, and the other underwent unrelated surgery.

Reference values. The results from the healthy controls were used to define reference values for normal pain threshold and P1 latency.

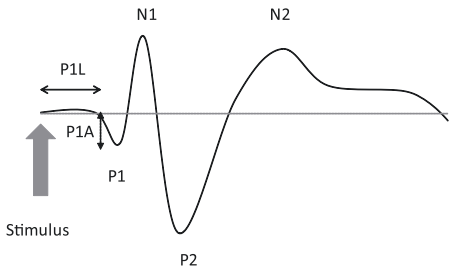


Fig. 1. Diagrammatic representation of rectal cortical evoked potential (CEP) components. Diagram shows positive and negative deflections following a stimulus. Neurophysiological convention describes negative potentials as upward deflections (N1 and N2) and positive potentials as downward deflections (P1 and P2). Latency refers to time (in ms) from stimulus trigger to peak component. Amplitude is defined as voltage difference between consecutive CEP peaks. Markers for P1 latency (P1L) and P1 amplitude (P1A) are shown.

Downloaded from ajpgi.physiology.org on September 13, 2011

Table 1. *Demographics of study participants*

	Controls (n = 21)	IBS Patients (n = 33)
Age, yr [mean (range)]	31.4 (21–46)	40.1 (20–63)
Sex, M/F [n (%)]	7/14 (33/67)	6/27 (18/82)
Type of IBS, n		
Diarrhea		16
Constipation		6
Alternating		8
Unclassified		3

Mean ages and sex distribution were similar in each group. Most irritable bowel syndrome (IBS) patients had diarrhea-predominant IBS.

Psychological Profile

Differences in anxiety between study groups faced with the same unknown testing situation were examined using the Spielberger State-Trait Anxiety Inventory (SSTAI), which measures state (SSTAI-state) and trait (SSTAI-trait) anxiety and overall anxiety levels. Scores range from 20 to 80, with higher scores indicating greater anxiety (29). Each questionnaire has 20 questions, with 4 possible Likert-type responses scored between 1 and 4. Scores >35 in either test were considered to be pathological.

Data Collection and Statistics

The RST and RPT (mA) are presented as means with 95% confidence intervals [means (95% CI)]. CEPs demonstrated characteristic morphology with P1, N1, P2, N2, and N3 peaks. The latency (ms) and amplitude (μV) of P1, P2, P3, N1, and N2 waves were measured for 75% RPT. Data are presented as mean differences between *visits 1, 2*, and *3*, with 95% CI to highlight the reproducibility of the measurements. Absolute values are also expressed as means (95% CI). As this was an exploratory study, we decided on sample number by considering the results of the NCCP disease model, where a sample size of 32 was sufficient to define phenotypes (16). One-way ANOVA was used to examine for inter- and intragroup differences between *visits 1, 2*, and *3*, and repeated-measures ANOVA assessed the repeatability of observations between visits. The mean 3-day difference between IBS patients and controls was analyzed by the Mann-Whitney test.

RESULTS

Demographics

Thirty-three Rome II IBS patients (27 female, mean age 40 yr) and 21 healthy controls (14 female, mean age 31 yr) were recruited. Demographic data are shown in Table 1. There were no significant differences in age and sex between the two groups.

Pain and Sensory Thresholds

RST and RPT. CONTROLS. At *visits 1, 2*, and *3,* RSTs [means (95% CI)] were 18.1 mA (15.2–20.9), 14.7 mA (12.8–16.7), and 15.8 mA (12.1–19.5), respectively, and RPTs were 70.2 mA (60.2–80.2), 71.61 mA (61.5–81.7), and 79.5 mA (69.3–89.6), respectively. Mean differences between *visits 1, 2*, and *3* are shown in Table 2. There were no statistical differences between *visits 1, 2*, and *3*.

IBS PATIENTS. At *visits 1, 2*, and *3,* RSTs were 18.7 mA (14.1–23.3), 21.3 mA (17.2–25.4), and 16.2 mA (12.6–19.9), respectively, and RPTs were 57.7 mA (48.5–66.9), 63.2 mA (53.2–73.2), and 58.2 mA (47.9–68.5), respectively. There were no significant differences between *visits 1, 2*, and *3* for the IBS patients (Table 2).

75% RPT. For 75% RPT, there were no differences between *visits 1, 2*, and *3* for the controls and IBS patients (data not shown).

IBS patients vs. controls. At *visit 2*, RST [mean (95% CI)] was 6.6 mA (1.4–11.7) lower for IBS patients than controls (P = 0.01; Fig. 2*A*). At *visit 3*, RPTs were significantly different between the IBS patients and controls, with a difference of −21.2 (−36.0 to −6.5) ($P < 0.01$; Fig. 2*B*). The mean 3-day RST was 16.2 mA (14.6–17.9) for controls and 18.8 mA (16.4–21.1) for the IBS patients; the difference was not significant. There was, however, a significant difference in RPT: 73.6 mA (68–79.2) for controls and 59.7 mA (54.2–65.1) for IBS patients ($P < 0.001$).

CEPs

CEP peak latency in controls. At *visit 1*, peak latencies were 73.5 ms (65.9–81.1) for P1, 119.9 ms (110.8–128.9) for N1, 224.2 ms (211.9–236.5) for P2, 397.4 ms (376.1–418.8) for N2, and 633.0 ms (589.4–676.6) for N3. The differences between *visits 1, 2*, and *3* are shown in Table 3. Only P2 latency was significantly different between *visits 1, 2*, and *3* (P = 0.04). P1 latency values for each subject at *visits 1, 2*, and *3* are shown in Fig. 3*A*.

CEP peak amplitudes in controls. At *visit 1,* peak amplitudes [mean (95% CI)] were 7.5 μV (5.5–9.6) for P1, 19.4 μV (14.5–24.2) for N1, 19.6 μV (15.5–23.7) for P2, and 7.9 μV (6.1–9.7) for N2. There was no statistically significant difference between the 3-day measurements (Table 3).

CEP peak latency in IBS patients. At *visit 1*, peak latencies [mean (95% CI)] were 96.4 ms (86.6–106.1) for P1, 138.8 ms (126.1–151.4) for N1, 239.2 ms (222.9–255.5) for P2, 405.8

Table 2. *Sensory and pain thresholds in controls and IBS patients*

	Visit 2 vs. *Visit 1*	*Visit 3* vs. *Visit 1*	*Visit 3* vs. *Visit 2*
Controls			
RST	−3.3 (−7.2 to 0.5)	−2.2 (−6.2 to 1.7)	1.1 (−2.9 to 5.1)
RPT	1.4 (−12.1 to 4.9)	9.2 (−4.4 to 22.9)	7.8 (−6.0 to 21.7)
75% RPT	0.13 (−10.8 to 11.1)	6.2 (−4.9 to 17.3)	6.1 (−5.2 to 17.3)
IBS patients			
RST	2.6 (−3.1 to 8.3)	−2.5 (−8.2 to 3.3)	−5.05 (−10.4 to 0.3)
RPT	5.5 (−7.8 to 18.8)	0.5 (−12.9 to 13.9)	−5.0 (−19.0 to 9.1)
75% RPT	4.7 (−6.3 to 15.6)	−0.3 (−11.3 to 10.7)	−5.0 (−16.4 to 6.4)

Values are means [95% confidence interval (CI)]. Data are shown as mean differences between IBS patients and controls for *visits 1, 2*, and *3*. There were no significant differences in rectal pain threshold (RPT) and rectal sensory threshold (RST) between *visits 1, 2*, and *3* in controls and IBS patients. Repeated-measures ANOVA was used for statistical analysis on participants who attended all 3 visits.

Downloaded from ajpgi.physiology.org on September 13, 2011

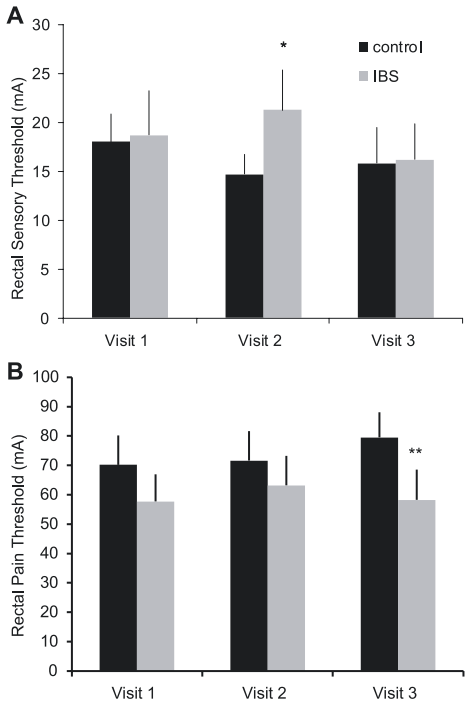


Fig. 2. Rectal sensory threshold (RST) and rectal pain threshold (RPT) for control subjects and patients with irritable bowel syndrome (IBS). *A*: RST was significantly greater [by 6.6 mA (95% CI 1.4–11.7)] for IBS patients than controls at *visit 2*. *$P = 0.01$. *B*: RPT was significantly less [by −21.2 mA (−36.0 to −6.5)] in IBS patients than controls at *visit 3*. **$P < 0.01$ (1-way ANOVA).

ms (375.6–436.0) for N2, and 674.3 ms (630.0–718.5) for N3. Differences between *visits 1, 2*, and *3* are shown in Table 4; the only significant difference between *visits 1, 2*, and *3* was for N2 latency ($P = 0.04$). Individual P1 latency values for *visits 1, 2*, and *3* are shown in Fig. 3*B*.

CEP peak amplitude in IBS patients. At *visit 1*, peak amplitude [mean (95% CI)] was 5.1 μV (3.4–6.9) for P1, 13.3 μV (9.5–17.0) for N1, 12.5 μV (9.4–15.7) for P2, and 5.3 μV (4.1–6.5) for N2. For P1, N1, and P2 amplitudes, there was no intervisit variation; N2 amplitude showed a significant difference between *visits 1, 2*, and *3* ($P = 0.02$; Table 4).

Comparative Profiles of IBS Patients and Controls

CEPs characteristics. LATENCY. Mean 3-day latencies [means (95% CI)] were longer for the IBS patients than controls for P1, N1, and P2 ($P < 0.001$): 101 ms (88.1–113.8) vs. 73.5 ms (69.3 - 77.6) for P1, 138.8 ms (130.6–147.0) vs. 116.1 ms (110.8–121.4) for N1, and 241.8 ms (232.2–251.3) vs. 219.7 ms (213.7–225.6) for P2. There was no significant difference for N2 latency between the two groups: 401.66 ms (388.1–415.3) and 388.3 ms (376.9–399.6) for IBS patients and controls, respectively ($P = 0.08$).

The mean differences in peak latencies between IBS patients and controls at *visits 1, 2*, and *3* are shown in Supplemental Table S1 (see Supplemental Material for this article, available online at the Journal website). N1 latency was consistently prolonged in the IBS patients compared with controls at *visits 1, 2*, and *3*. In addition, P1 latency was significantly longer in the IBS patients at *visits 1* and *2*, and P2 latency was prolonged in the IBS patients at *visits 2* and *3*.

Amplitude. Comparison of 3-day mean amplitudes revealed lower amplitudes [mean (95% CI)] for the IBS patients than controls for the four components ($P < 0.005$): 5.7 μV (4.7–6.7) and 7.7 μV (6.5–9.0) for P1, 14.2 μV (12.1–16.2) and 21.4 μV (18.4–24.5) for N1, 14.1 μV (12.3–15.9) and 22.5 μV (20.0–25.0) for P2, and 6.3 μV (5.6–7.1) and 8.5 μV (7.4–9.7) for N2 for the IBS patients and controls, respectively. When P1 amplitudes in IBS patients and controls were comparable for each visit, no significant differences were noted. N1 and P2 amplitudes were consistently lower at *visits 1, 2*, and *3* for the IBS patients than controls. N2 amplitude was only significantly lower at *visit 1* (see Supplemental Table S1).

Psychological Profile

SSTAI-state scores [means (95% CI)] for the IBS patients were 38.9 (35.4–42.3), 37.6 (33.8–41.5), and 39.7 (34.6–44.8) for *visits 1*, *2*, and *3*, respectively. In comparison, the scores for controls were 31.9 (28.8–35.0), 28.9 (26.3–31.6), and 31.6 (26.9–36.4). For each group, there were no significant interday differences for these scores (Fig. 4*A*; see Supplemental Table S2).

Table 3. *Mean differences between visits 1, 2, and 3 for CEP components in controls*

	Visit 2 vs. *Visit 1*	*Visit 3* vs. *Visit 1*	*Visit 3* vs. *Visit 2*	*P* Value
Latency				
P1 (*peak 1*)	0.6 (−9.7 to 11.0)	−0.8 (−11.1 to 9.6)	−1.4 (−12.0 to 9.2)	NS
N1 (*peak 2*)	−5.1 (−18.1 to 7.8)	−6.6 (−19.5 to 6.4)	−1.4 (−15.0 to 12.1)	NS
P2 (*peak 3*)	−5.3 (−19.8 to 9.3)	−8.7 (−23.3 to 5.9)	−3.4 (−16.8 to 9.9)	0.04
N2 (*peak 4*)	−11.7 (−39.4 to 6.0)	−16.8 (−44.6 to 10.9)	−5.1 (−32.7 to 22.4)	NS
Amplitude				
P1 (*peak 1*)	0.6 (−2.5 to 3.7)	−0.1 (−3.1 to 3.0)	−0.7 (−4.0 to 2.7)	NS
N1 (*peak 2*)	2.7 (−4.8 to 10.1)	3.8 (−3.7 to 11.2)	1.1 (−7.0 to 9.2)	NS
P2 (*peak 3*)	3.5 (−2.5 to 9.5)	5.6 (−0.4 to 11.5)	2.0 (−4.3 to 8.4)	NS
N2 (*peak 4*)	0.5 (−2.4 to 3.4)	1.5 (−1.4 to 4.4)	1.0 (−2.2 to 4.2)	NS

Values are means (95% CI); $n = 21$. Five cortical evoked potentials (CEPs) were observed. There were no significant differences between amplitude components at *visits 1, 2,* and *3*. Repeated-measures ANOVA was used for statistical analysis on participants who attended all 3 visits ($n = 19$). There was a significant difference in P2 latency between visits ($P = 0.04$). NS, not significant.

Downloaded from ajpgi.physiology.org on September 13, 2011

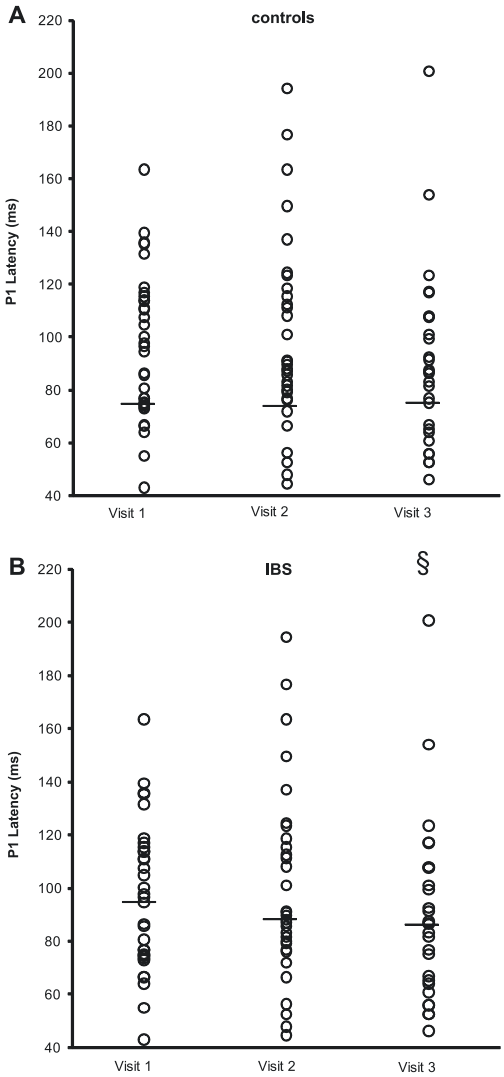


Fig. 3. Individual data points for P1 latency at *visits 1, 2,* and *3* for controls (*A*) and IBS patients (*B*). §P1 latency = 608.6 ms.

SSTAI-trait scores [means (95% CI)] for the IBS patients were 44.5 (40.7–48.3), 40.7 (36.7–44.7), and 44.0 (39.3–48.7) at *visits 1, 2*, and *3*, respectively. Comparative scores for controls were 33.9 (30.8–37.0), 32.6 (29.3–35.9), and 32.2 (28.6–35.8) (Fig. 4*B*). There were no differences in the scores at *visits 1, 2,* and *3* for each group (see Supplemental Table S2).

IBS patients vs. controls. At each visit, state and trait scores were significantly greater for IBS patients than controls. This suggests that IBS patients mount a greater anxiety response to the same perceived threat of electrical stimulation that does not diminish over time with increasing familiarity of the test protocol.

Reference Values to Identify Phenotypes

Healthy controls were used to define the control reference values for P1 latency. The 3-day mean was 73.4 ms with a standard deviation (SD) of 16.1 ms. Therefore, P1 latencies >106 ms (2 SDs above the reference mean P1 latency) were considered abnormal. Hypersensitivity to rectal stimulation was defined as RPT ≤50 mA, as all healthy controls had levels above this value (range for RPT was 50–92).

IBS Phenotypes: Subgroup Analysis

Low (≤50 mA) RPT. In 12 IBS patients, mean 3-day RPT was ≤50 mA. P1 latency was >106 ms in four of these IBS patients and ≤106 ms in eight IBS patients. Eight of these IBS patients had visceral hypersensitivity, as defined by RPT <50 mA, with a normal-latency P1 ≤106 ms, and four showed features of hypervigilance, with low pain thresholds (≤50 mA) and high P1 latency (>106 ms; Figs. 5 and 6).

Normal (>50 mA) RPT. A 3-day mean pain threshold >50 mA was observed in 21 IBS patients. Five of these IBS patients exhibited prolonged (>106 ms) latency, and 16 exhibited normal latency. Hyposensitivity, as defined by high or normal pain threshold >50 mA and prolonged P1 latency >106 ms, was therefore noted in five IBS patients. The greatest proportion of IBS patients (49%) showed normal RPT >50 mA and normal P1 latency (Figs. 5 and 6).

Normal, hypervigilant, and hypersensitive profiles. The difference between CEP, pain threshold, and anxiety state emphasizing the above profiles is shown for three subjects in Supplemental Fig. S1.

DISCUSSION

This study is the first to use rectal CEP over time to examine IBS neurophysiological profiles. We show that rectal CEPs generate a reproducible, objective assessment of rectal afferent sensitivity and are consistent for interday testing in healthy controls and IBS patients. The rectal CEP recordings were also morphologically similar between *visits 1, 2,* and *3* for the four major components of the early primary afferent cortical response.

CEP Interpretation

We, and others, previously showed that the early CEP complex in response to visceral stimulation is stimulus-dependent and represents activation of the primary or secondary somatosensory cortex and insula (15). In esophageal studies, cortical activity after ~150 ms following electrical stimulation relates to brain endogenous processing of afferent information. Unlike early CEP components, which depend on the intensity of the transmitted afferent signal strength, later components appear to represent a more general arousal-type response to the stimulus. These later responses may be heightened in response to a low level of stimulation if a subject's arousal or attention is altered and may provide an objective measure of hypervigilance.

Downloaded from ajpgi.physiology.org on September 13, 2011

Table 4. *CEP components in IBS patients*

	Visit 2 vs. *Visit 1*	*Visit 3* vs. *Visit 1*	*Visit 3* vs. *Visit 2*	*P* Value
Latency				
P1 (*peak 1*)	3.2 (−28.0 to 34.3)	11.4 (−20.3 to 43.2)	8.3 (−30.7 to 47.3)	NS
N1 (*peak 2*)	4.9 (−15.0 to 24.7)	−5.2 (−25.3 to 15.0)	−10.0 (−31.8 to 11.7)	NS
P2 (*peak 3*)	3.9 (−19.3 to 27.1)	4.2 (−19.5 to 27.8)	0.3 (−24.1 to 24.7)	NS
N2 (*peak 4*)	−3.3 (−36.4 to 29.8)	−9.7 (−43.4 to 24.0)	−6.3 (−34.1 to 21.4)	0.04
Amplitude				
P1 (*peak 1*)	0.9 (−1.6 to 3.4)	0.9 (−1.6 to 3.5)	0.02 (−2.6 to 2.6)	NS
N1 (*peak 2*)	1.2 (−3.8 to 6.2)	1.6 (−3.5 to 6.7)	0.4 (−4.6 to 5.4)	NS
P2 (*peak 3*)	1.7 (−2.6 to 6.0)	3.4 (−1.0 to 7.7)	1.7 (−2.7 to 6.1)	NS
N2 (*peak 4*)	1.0 (−0.7 to 2.8)	2.2 (0.4 to 4.0)	1.2 (−0.7 to 3.0)	0.02

Values [means (95% CI)] are mean differences between *visits 1, 2*, and *3* for each cortical evoked potential (CEP) component ($n = 33$). Five potentials were observed. There were significant differences in N2 latency and N2 amplitude between *visits 1, 2,* and *3* in IBS patients. Repeated-measures ANOVA was used for statistical analysis on participants who attended all 3 visits ($n = 29$).

P1 latency represents the time interval between stimulation and activation of these sensory discriminatory brain regions. In healthy subjects, as the perceived stimulus intensity increases, there is a concurrent reduction in latency and an increase in amplitude of the CEP components (14). This provides an objective measure that correlates strongly with a subjective end point. In our previous NCCP work, we identified a group of patients expressing pain at relatively low stimulation levels but with normal P1 latency (16), indicative of afferent sensitization. A second group that also reported pain at low stimulation intensities showed prolonged-latency P1 responses. As these responses were similar to those expected when CEPs were acquired around the subject's perception threshold, we surmised that the afferent signaling pathway was not sensitized; instead, the subjects were amplifying normal-intensity stimuli once these stimuli had reached the brain, with hypervigilance being a possible process by which this could happen. We acknowledge that stress-induced rectal hyperalgesia may also be a mechanism by which this kind of central amplification may occur, but until this is proven, the term "hypervigilance" has been used to distinguish between these two groups.

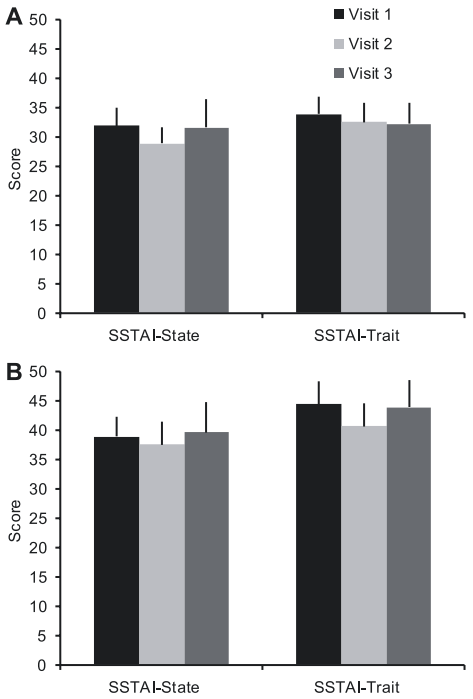


Fig. 4. Spielberger State and Trait Anxiety Index (SSTAI-state and SSTAI-trait) scores [mean (95% CI)] at *visits 1, 2*, and *3* in controls (*A*) and IBS patients (*B*). Score was <35 (the level for diagnosis of anxiety) in all controls. There were no significant differences between *visits 1, 2*, and *3*. Scores were higher (>35) for IBS patients than controls, but there were no differences between *visits 1, 2*, and *3*.

CEP as a Cortical Sensory Processing Tool

CEP techniques have been used by neurophysiologists to investigate visual, auditory, and somatosensory pathways, leading to adaptations to study visceral sensation (3, 9). In rectal studies, different visceral stimuli, namely, electrical and mechanical modalities, generated identical CEP waveforms, except for a slight latency difference due to a 50-ms delay in the balloon inflation (11). We chose an electric modality, because it provides distinct painful stimulation, which may be more applicable in pain studies. Rectal distension responses are mediated by unmyelinated C-fibers and thinly myelinated Aδ-fibers through the pelvic nerve (19, 24, 28). A previous study

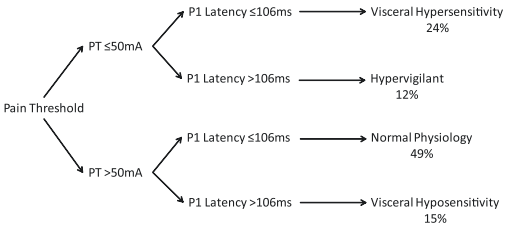


Fig. 5. Definition of profiles according to pain thresholds (PT) and P1 latency. Patients with RPT ≤50 mA were considered hypersensitive. Hypersensitive group was further subclassified according to P1 latency, which represents time taken for stimulus to reach cortex; 24% had latency ≤106 ms, indicative of true visceral hypersensitivity. Those with a prolonged latency were hypervigilant. Within the group with normal rectal sensation, 15% showed prolonged latency, indicative of hyposensitivity.

Downloaded from ajpgi.physiology.org on September 13, 2011

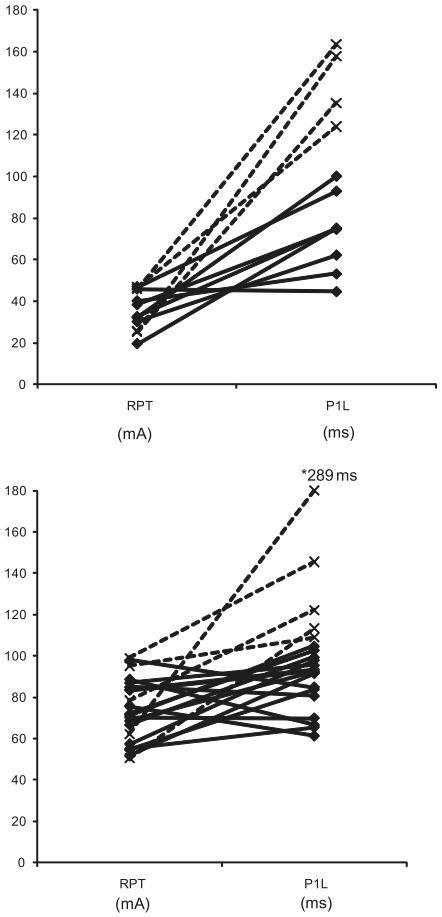


Fig. 6. Profile for each IBS patient determined by RPT-P1 latency relationship. *Top*: 3-day mean results for patients with RPT ≤50 mA. In 4 patients, P1 latency was >106 ms, indicative of hypervigilance; in remaining 8 patients, P1 latency was ≤106 ms, indicative of visceral hypersensitivity. *Bottom*: 3-day mean results for patients with RPT >50 mA. Only 5 patients showed a prolonged (>106 ms) P1 latency, indicative of hyposensitivity; remaining 16 patients showed a normal P1 latency. *Very high (289 ms) P1 latency in 1 patient.

showed shorter-than-expected CEP latencies for C-fiber stimulation (2), suggesting that electrical and mechanical rectal CEPs are mediated via Aδ-fibers.

Rectal CEP morphology exhibits some differences from CEP morphology of other gut segments; for example, P1 latency is shorter than duodenal and esophageal CEP latency (13, 17). The reason for the shorter P1 latency is unclear; further studies on conduction velocities may clarify whether this may represent faster-conducting afferent pathways conveying rectal sensation, reflecting prominence given to continence mechanisms.

CEPs have sufficient temporal resolution to discriminate between primary (stimulus-specific cortical response) and secondary (non-stimulus-specific cortical response) processing of visceral afferent activity. The latter contains endogenous pain components affected by attention, cognition, and behavior. This distinction is important, as it differentiates primary visceral hypersensitivity (increased CEP amplitude for a given stimulus) from competing delayed central contributions influenced by factors such as psychological state. Previous research on esophageal cortical sensory processing revealed that evoked potential (EP) activity up to 150 ms after stimulation represents the primary component (14–16). Activity occurring after >250 ms represents endogenous cortical activity, as observed by measurement of EP latency to anticipated esophageal stimulus (17). Studying visceral sensation with CEPs should identify whether IBS is driven by primary visceral hypersensitivity or psychological hypersensitivity.

Drewes et al. (6) took this concept of temporal resolution further by incorporating regional brain activation to map networks involved in EPs to sensory stimuli. Activity from EEGs in response to painful esophageal stimuli showed sequential activation of the thalamus, insula, cingulate, and somatosensory cortex. Using grouped CEP responses, Drewes et al. also showed subtle differences in the cingulate cortex activation region in IBS patients. Different cortical topography may explain partly the differences in latency and amplitude between various CEP components between IBS patients and controls in our study.

CEP Components Identify Distinct IBS Phenotypes

Individual components of rectal CEP were used to define normal population reference ranges for subgroup analysis in our IBS population to explore the prevalence of primary visceral hypersensitivity. We found that IBS represents a heterogeneous patient group with different neurophysiological profiles. Most patients with IBS (49%) had a normal pain threshold and normal sensory afferent transmission. Only a minority of IBS patients (24%) had afferent visceral hypersensitivity with pain threshold <50 mA and normal latency. A similar number showed hyposensitivity or hypervigilance, with a low pain threshold and increased latency.

Pain thresholds in IBS patients were significantly different from those in controls at *visit 3*. This was mainly due to habituation of RPT over time in controls, whereas values for IBS patients remained consistent. Habituation to painful stimulation is mediated via descending inhibitory mechanisms, which have been shown to be defective in IBS patients in several studies (23, 32). This might suggest that the treatment strategy for attenuating visceral afferent transmission (via analgesics) would differ from boosting endogenous pain mechanisms, for example, endogenous opioids, such as β-endorphin. A recent preclinical study with probiotics showed that attenuation of experimental visceral hypersensitivity was associated with upregulation of peripheral opioid receptors (30).

In previous work from our department, Murray et al. (22) demonstrated little difference in sensory thresholds between IBS patients and controls until experimental stress was applied (22). The wide range of sensory response from hyper- to hyposensitivity seen in our cohort most likely skews the sensory threshold data upward. Pain thresholds differed be-

Downloaded from ajpgi.physiology.org on September 13, 2011

tween controls and IBS patients only because of an increase in pain threshold in controls over time; this appears to be attenuated in some IBS patients.

We also looked at potential confounders, such as state and trait anxiety. Of the five studies that previously used SSTAI for IBS patients, only three recorded mean SSTAI-state scores with an overall mean of 47.7 (8). Only one study recorded a mean SSTAI-state score of 35.5 for a control group. The mean SSTAI-trait score was 47.2 for IBS patients (in 4 studies) and 41.2 for controls (in 2 studies) (8). The SSTAI-trait score is more time-independent than the SSTAI-state score, but both indexes showed little variation in either group over *visits 1, 2,* and *3*. At no point did the state score drop below the case threshold of 35 in the IBS group.

Conclusions

This is the first study to validate CEP testing in IBS. Testing was reproducible and robust. Early CEP responses and pain thresholds defined distinct neurophysiological groups, supporting the concept of IBS as a heterogeneous syndrome. Local and central mechanisms are likely to contribute to each profile; defining each contribution may allow effective drug therapy. The abundant CEP data generated from this study can be used in future therapeutic studies.

GRANTS

This work was funded by an educational grant from GlaxoSmithKline Research and Development.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

1. **Bernstein CN, Frankenstein UN, Rawsthorne P, Pitz M, Summers R, McIntyre MC.** Cortical mapping of visceral pain in patients with GI disorders using functional magnetic resonance imaging. *Am J Gastroenterol* 97: 319–327, 2002.
2. **Bromm B, Treede RD.** Human cerebral potentials evoked by CO_2 laser stimuli causing pain. *Exp Brain Res* 67: 153–162, 1987.
3. **Collet L, Meunier P, Duclaux R, Chery-Croze S, Falipou P.** Cerebral evoked potentials after endorectal mechanical stimulation in humans. *Am J Physiol Gastrointest Liver Physiol* 254: G477–G482, 1988.
4. **Dorn SD, Palsson OS, Thiwan SI, Kanazawa M, Clark WC, van Tilburg MA, Drossman DA, Scarlett Y, Levy RL, Ringel Y, Crowell MD, Olden KW, Whitehead WE.** Increased colonic pain sensitivity in irritable bowel syndrome is the result of an increased tendency to report pain rather than increased neurosensory sensitivity. *Gut* 56: 1202–1209, 2007.
5. **Drewes AM, Gregersen H.** Multimodal pain stimulation of the gastrointestinal tract. *World J Gastroenterol* 12: 2477–2486, 2006.
6. **Drewes AM, Sami SA, Dimcevski G, Nielsen KD, Funch-Jensen P, Valeriani M, Arendt-Nielsen L.** Cerebral processing of painful oesophageal stimulation: a study based on independent component analysis of the EEG. *Gut* 55: 619–629, 2006.
7. **Drossman DA.** Introduction: The Rome Foundation and Rome III. *Neurogastroenterol Motil* 19: 783–786, 2007.
8. **Elliott C, Kamm MA, Bullas D, Gurmany S, Arebi N.** High depression and anxiety scores in a systematic review of psychological questionnaires in irritable bowel syndrome (Abstract). *Gut* 58 Suppl II: A185, 2010.
9. **Frieling T, Enck P, Wienbeck M.** Cerebral responses evoked by electrical stimulation of rectosigmoid in normal subjects. *Dig Dis Sci* 34: 202–205, 1989.
10. **Fukudo S, Nomura T, Muranaka M, Taguchi F.** Brain-gut response to stress and cholinergic stimulation in irritable bowel syndrome. A preliminary study. *J Clin Gastroenterol* 17: 133–141, 1993.
11. **Harris ML, Hobson AR, Hamdy S, Thompson DG, Akkermans LM, Aziz Q.** Neurophysiological evaluation of healthy human anorectal sensation. *Am J Physiol Gastrointest Liver Physiol* 291: G950–G958, 2006.
12. **Hobday DI, Hobson A, Furlong PL, Thompson DG, Aziz Q.** Comparison of cortical potentials evoked by mechanical and electrical stimulation of the rectum. *Neurogastroenterol Motil* 12: 547–554, 2000.
13. **Hobday DI, Hobson AR, Sarkar S, Furlong PL, Thompson DG, Aziz Q.** Cortical processing of human gut sensation: an evoked potential study. *Am J Physiol Gastrointest Liver Physiol* 283: G335–G339, 2002.
14. **Hobson AR, Sarkar S, Furlong PL, Thompson DG, Aziz Q.** A cortical evoked potential study of afferents mediating human esophageal sensation. *Am J Physiol Gastrointest Liver Physiol* 279: G139–G147, 2000.
15. **Hobson AR, Furlong PL, Worthen SF, Hillebrand A, Barnes GR, Singh KD, Aziz Q.** Real-time imaging of human cortical activity evoked by painful esophageal stimulation. *Gastroenterology* 128: 610–619, 2005.
16. **Hobson AR, Furlong PL, Sarkar S, Matthews PJ, Willert RP, Worthen SF, Unsworth BJ, Aziz Q.** Neurophysiologic assessment of esophageal sensory processing in noncardiac chest pain. *Gastroenterology* 130: 80–88, 2006.
17. **Hollerbach S, Fitzpatrick D, Shine G, Kamath MV, Upton AR, Tougas G.** Cognitive evoked potentials to anticipated oesophageal stimulus in humans: quantitative assessment of the cognitive aspects of visceral perception. *Neurogastroenterol Motil* 11: 37–46, 1999.
18. **Kern MK, Shaker R.** Cerebral cortical registration of subliminal visceral stimulation. *Gastroenterology* 122: 290–298, 2002.
19. **Mayer EA, Raybould HE.** Role of visceral afferent mechanisms in functional bowel disorders. *Gastroenterology* 99: 1688–1704, 1990.
20. **Mertz H, Naliboff B, Munakata J, Niazi N, Mayer EA.** Altered rectal perception is a biological marker of patients with irritable bowel syndrome. *Gastroenterology* 109: 40–52, 1995.
21. **Munakata J, Naliboff B, Harraf F, Kodner A, Lembo T, Chang L, Silverman DH, Mayer EA.** Repetitive sigmoid stimulation induces rectal hyperalgesia in patients with irritable bowel syndrome. *Gastroenterology* 112: 55–63, 1997.
22. **Murray CD, Flynn J, Ratcliffe L, Jacyna MR, Kamm MA, Emmanuel AV.** Effect of acute physical and psychological stress on gut autonomic innervation in irritable bowel syndrome. *Gastroenterology* 127: 1695–1703, 2004.
23. **Naliboff BD, Berman S, Suyenobu B, Labus JS, Chang L, Stains J, Mandelkern MA, Mayer EA.** Longitudinal change in perceptual and brain activation response to visceral stimuli in irritable bowel syndrome patients. *Gastroenterology* 131: 352–365, 2006.
24. **Ness TJ, Metcalf AM, Gebhart GF.** A psychophysiological study in humans using phasic colonic distension as a noxious visceral stimulus. *Pain* 43: 377–386, 1990.
25. **Price DD, Zhou Q, Moshiree B, Robinson ME, Verne GN.** Peripheral and central contributions to hyperalgesia in irritable bowel syndrome. *J Pain* 7: 529–535, 2006.
26. **Sarkar S, Aziz Q, Woolf CJ, Hobson AR, Thompson DG.** Contribution of central sensitisation to the development of non-cardiac chest pain. *Lancet* 356: 1154–1159, 2000.
27. **Sarkar S, Hobson AR, Furlong PL, Woolf CJ, Thompson DG, Aziz Q.** Central neural mechanisms mediating human visceral hypersensitivity. *Am J Physiol Gastrointest Liver Physiol* 281: G1196–G1202, 2001.
28. **Sengupta JN, Gebhart GF.** Characterization of mechanosensitive pelvic nerve afferent fibers innervating the colon of the rat. *J Neurophysiol* 71: 2046–60, 1994.
29. **Spielberger CD, Gorsuch RL, Lushene RE.** *Manual for the State-Trait Anxiety Inventory.* Palo Alto, CA: Consulting Psychologist Press, 1970.
30. **Verdu EF, Bercik P, Verma-Gandhu M, Huang XX, Blennerhassett P, Jackson W, Mao Y, Wang L, Rochat F, Collins SM.** Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. *Gut* 55: 182–90, 2006.
31. **Whitehead WE, Palsson OS.** Is rectal pain sensitivity a biological marker for irritable bowel syndrome: psychological influences on pain perception. *Gastroenterology* 115: 1263–71, 1998.
32. **Wilder-Smith CH, Schindler D, Lovblad K, Redmond SM, Nirkko A.** Brain functional magnetic resonance imaging of rectal pain and activation of endogenous inhibitory mechanisms in irritable bowel syndrome patient subgroups and healthy controls. *Gut* 53: 1595–601, 2004.
33. **Yaguez L, Coen S, Gregory LJ, Amaro E Jr, Altman C, Brammer MJ, Bullmore ET, Williams SC, Aziz Q.** Brain response to visceral aversive conditioning: a functional magnetic resonance imaging study. *Gastroenterology* 128: 1819–29, 2005.

Downloaded from ajpgi.physiology.org on September 13, 2011

REFERENCES

1. Neal KR, Barker L, Spiller RC. Prognosis in post-infective irritable bowel syndrome: A six year follow up study. British Medical Journal 2002;51(3):410.

2. Spiegel BM, Gralnek IM, Bolus R, Chang L, Dulai GS, Naliboff B, Mayer EA. Is a negative colonoscopy associated with reassurance or improved health-related quality of life in irritable bowel syndrome? Gastrointest Endosc 2005, Dec;62(6):892-9.

3. Wilson S, Roberts L, Roalfe A, Bridge P, Singh S. Prevalence of irritable bowel syndrome: A community survey. Br J Gen Pract 2004, Jul;54(504):495-502.

4. Drossman DA, Li Z, Andruzzi E, Temple RD, Talley NJ, Thompson WG, et al. U.S. Householder survey of functional gastrointestinal disorders. Prevalence, sociodemography, and health impact. Dig Dis Sci 1993, Sep;38(9):1569-80.

5. O'Keefe E, Talley NJ. Irritable bowel syndrome in the elderly. Clin Geriatr Med 1991, May;7(2):265-86.

6. Bennett G, Talley NJ. Irritable bowel syndrome in the elderly. Best Practice & Research Clinical Gastroenterology 2002;16(1):63-76.

7. Talley NJ, O'Keefe EA, Zinsmeister AR, Melton LJ. Prevalence of gastrointestinal symptoms in the elderly: A population-based study. Gastroenterology 1992, Mar;102(3):895-901.

8. Talley NJ, Zinsmeister AR, Van Dyke C, Melton LJ. Epidemiology of colonic symptoms and the irritable bowel syndrome. Gastroenterology 1991, Oct;101(4):927-34.

9. van der Horst HE, van Dulmen AM, Schellevis FG, van Eijk JT, Fennis JF, Bleijenberg G. Do patients with irritable bowel syndrome in primary care really differ from outpatients with irritable bowel syndrome? Gut 1997, Nov;41(5):669-74.

10. Simrén M, Abrahamsson H, Svedlund J, Björnsson ES. Quality of life in patients with irritable bowel syndrome seen in referral centers versus primary care: The impact of gender and predominant bowel pattern. Scand J Gastroenterol 2001, May;36(5):545-52.

11. Corney RH, Stanton R. Physical symptom severity, psychological and social dysfunction in a series of outpatients with irritable bowel syndrome. J Psychosom Res 1990;34(5):483-91.

12. Lee OY, Mayer EA, Schmulson M, Chang L, Naliboff B. Gender-Related differences in IBS symptoms. Am J Gastroenterol 2001, Jul;96(7):2184-93.

13. Heitkemper MM, Cain KC, Jarrett ME, Burr RL, Hertig V, Bond EF. Symptoms across the menstrual cycle in women with irritable bowel syndrome. Am J Gastroenterol 2003, Feb;98(2):420-30.

14. Crowell MD, Dubin NH, Robinson JC, Cheskin LJ, Schuster MM, Heller BR, Whitehead WE. Functional bowel disorders in women with dysmenorrhea. Am J Gastroenterol 1994, Nov;89(11):1973-7.

15. Houghton LA, Lea R, Jackson N, Whorwell PJ. The menstrual cycle affects rectal sensitivity in patients with irritable bowel syndrome but not healthy volunteers. Gut 2002, Apr;50(4):471-4.

16. Wigington WC, Johnson WD, Minocha A. Epidemiology of irritable bowel syndrome among african americans as compared with whites: A population-based study. Clin Gastroenterol Hepatol 2005, Jul;3(7):647-53.

17. Minocha A, Johnson WD, Wigington WC. Prevalence of abdominal and pelvic surgeries in patients with irritable bowel syndrome: Comparison between caucasian and african americans. Am J Med Sci 2008, Feb;335(2):82-8.

18. Ng B, Dimsdale JE, Rollnik JD, Shapiro H. The effect of ethnicity on prescriptions for patient-controlled analgesia for post-operative pain. Pain 1996, Jul;66(1):9-12.

19. Dimsdale JE. Stalked by the past: The influence of ethnicity on health. Psychosom Med 2000;62(2):161-70.

20. Hahn BA, Yan S, Strassels S. Impact of irritable bowel syndrome on quality of life and resource use in the united states and united kingdom. Digestion 1999, Jan;60(1):77-81.

21. Akehurst RL, Brazier JE, Mathers N, O'Keefe C, Kaltenthaler E, Morgan A, et al. Health-Related quality of life and cost impact of irritable bowel syndrome in a UK primary care setting. Pharmacoeconomics 2002;20(7):455-62.

22. Gralnek IM, Hays RD, Kilbourne A, Naliboff B, Mayer EA. The impact of irritable bowel syndrome on health-related quality of life. Gastroenterology 2000, Sep;119(3):654-60.

23. Creed F, Ratcliffe J, Fernandez L, Tomenson B, Palmer S, Rigby C, et al. Health-Related quality of life and health care costs in severe, refractory irritable bowel syndrome. Ann Intern Med 2001, May 1;134(9 Pt 2):860-8.

24. Spiegel BM, Gralnek IM, Bolus R, Chang L, Dulai GS, Mayer EA, Naliboff B. Clinical determinants of health-related quality of life in patients with irritable bowel syndrome. Arch Intern Med 2004, Sep 13;164(16):1773-80.

25. Hungin AP, Whorwell PJ, Tack J, Mearin F. The prevalence, patterns and impact of irritable bowel syndrome: An international survey of 40,000 subjects. Aliment Pharmacol Ther 2003, Mar 1;17(5):643-50.

26. Cain KC, Jarrett ME, Burr RL, Rosen S, Hertig VL, Heitkemper MM. Gender differences in gastrointestinal, psychological, and somatic symptoms in irritable bowel syndrome. Dig Dis Sci 2008, Nov 1.

27. Koloski NA, Talley NJ, Boyce PM. Does psychological distress modulate functional gastrointestinal symptoms and health care seeking? A prospective, community cohort study. Am J Gastroenterol 2003, Apr;98(4):789-97.

28. Longstreth GF, Yao JF. Irritable bowel syndrome and surgery: A multivariable analysis. Gastroenterology 2004, Jun;126(7):1665-73.

29. Kennedy TM, Jones RH. Epidemiology of cholecystectomy and irritable bowel syndrome in a UK population. Br J Surg 2000, Dec;87(12):1658-63.

30. Miller V, Hopkins L, Whorwell PJ. Suicidal ideation in patients with irritable bowel syndrome. Clin Gastroenterol Hepatol 2004, Dec;2(12):1064-8.

31. Spiegel B, Schoenfeld P, Naliboff B. Systematic review: The prevalence of suicidal behaviour in patients with chronic abdominal pain and irritable bowel syndrome. Aliment Pharmacol Ther 2007, Jul 15;26(2):183-93.

32. Maxion-Bergemann S, Thielecke F, Abel F, Bergemann R. Costs of irritable bowel syndrome in the UK and US. Pharmacoeconomics 2006;24(1):21-37.

33. sandler RS, Everhart JE, Donowitz M, Adams E, Cronin K, Goodman C, et al. The burden of selected digestive diseases in the united states. Gastroenterology 2002;122(5):1500-11.

34. Bentkover JD, Field C, Greene EM, Plourde V, Casciano JP. The economic burden of irritable bowel syndrome in canada. Can J Gastroenterol 1999, Mar;13 Suppl A:89A-96A.

35. Sandler RS, Everhart JE, Donowitz M, Adams E, Cronin K, Goodman C, et al. The burden of selected digestive diseases in the united states. Gastroenterology 2002, May;122(5):1500-11.

36. Drossman DA. The functional gastrointestinal disorders and the rome III process. Gastroenterology 2006;130(5):1377-90.

37. Kruis W, Thieme C, Weinzierl M, Schüssler P, Holl J, Paulus W. A diagnostic score for the irritable bowel syndrome. Its value in the exclusion of organic disease. Gastroenterology 1984, Jul;87(1):1-7.

38. Manning AP, Thompson WG, Heaton KW, Morris AF. Towards positive diagnosis of the irritable bowel. Br Med J 1978, Sep 2;2(6138):653-4.

39. Mearin X Bad& x 000 ED a A Balboa F. Irritable bowel syndrome prevalence varies enormously depending on the employed diagnostic criteria: Comparison of rome II versus previous criteria in a general population. Scand J Gastroenterol 2001;36(11):1155-61.

40. Boyce PM, Koloski NA, Talley NJ. Irritable bowel syndrome according to varying diagnostic criteria: Are the new rome II criteria unnecessarily restrictive for research and practice? Am J Gastroenterol 2000, Nov;95(11):3176-83.

41. Sperber AD, Shvartzman P, Friger M, Fich A. Unexpectedly low prevalence rates of IBS among adult israeli jews. Neurogastroenterol Motil 2005, Apr;17(2):207-11.

42. Hammer J, Eslick GD, Howell SC, Altiparmak E, Talley NJ. Diagnostic yield of alarm features in irritable bowel syndrome and functional dyspepsia. Gut 2004, May;53(5):666-72.

43. Vanner SJ, Depew WT, Paterson WG, DaCosta LR, Groll AG, Simon JB, Djurfeldt M. Predictive value of the rome criteria for diagnosing the irritable bowel syndrome. Am J Gastroenterol 1999, Oct;94(10):2912-7.

44. Stewart M, Brown JB, Donner A, McWhinney IR, Oates J, Weston WW, Jordan J. The impact of patient-centered care on outcomes. J Fam Pract 2000, Sep;49(9):796-804.

45. Roter DL, Hall JA, Merisca R, Nordstrom B, Cretin D, Svarstad B. Effectiveness of interventions to improve patient compliance: A meta-analysis. Med Care 1998, Aug;36(8):1138-61.

46. Drossman DA, Morris CB, Hu Y, Toner BB, Diamant N, Leserman J, et al. A prospective assessment of bowel habit in irritable bowel syndrome in women: Defining an alternator. Gastroenterology 2005, Mar;128(3):580-9.

47. Garrigues V, Mearin F, Badía X, Balboa A, Benavent J, Caballero A, et al. Change over time of bowel habit in irritable bowel syndrome: A prospective, observational, 1-year follow-up study (RITMO study). Aliment Pharmacol Ther 2007, Feb 1;25(3):323-32.

48. Waller SL, Misiewicz JJ. Prognosis in the irritable-bowel syndrome. A prospective study. Lancet 1969, Oct 11;2(7624):754-6.

49. Talley NJ, Weaver AL, Zinsmeister AR, Melton LJ. Onset and disappearance of gastrointestinal symptoms and functional gastrointestinal disorders. Am J Epidemiol 1992, Jul 15;136(2):165-77.

50. Lembo T, Fullerton S, Diehl D, Raeen H, Munakata J, Naliboff B, Mayer EA. Symptom duration in patients with irritable bowel syndrome. Am J Gastroenterol 1996, May;91(5):898-905.

51. Locke GR, Zinsmeister AR, Fett SL, Melton LJ, Talley NJ. Overlap of gastrointestinal symptom complexes in a US community. Neurogastroenterol Motil 2005, Feb;17(1):29-34.

52. Agréus L, Svärdsudd K, Nyrén O, Tibblin G. Irritable bowel syndrome and dyspepsia in the general population: Overlap and lack of stability over time. Gastroenterology 1995, Sep;109(3):671-80.

53. Halder SL, Locke GR, Schleck CD, Zinsmeister AR, Melton LJ, Talley NJ. Natural history of functional gastrointestinal disorders: A 12-year longitudinal population-based study. Gastroenterology 2007, Sep;133(3):799-807.

54. Whorwell PJ, McCallum M, Creed FH, Roberts CT. Non-Colonic features of irritable bowel syndrome. Gut 1986, Jan;27(1):37-40.

55. Whitehead WE, Palsson O, Jones KR. Systematic review of the comorbidity of irritable bowel syndrome with other disorders: What are the causes and implications? Gastroenterology 2002, Apr;122(4):1140-56.

56. MacDonald AJ, Bouchier IA. Non-Organic gastrointestinal illness: A medical and psychiatric study. Br J Psychiatry 1980, Mar;136:276-83.

57. Colgan S, Creed F, Klass H. Symptom complaints, psychiatric disorder and abnormal illness behaviour in patients with upper abdominal pain. Psychol Med 1988, Nov;18(4):887-92.

58. Craig TK, Brown GW. Goal frustration and life events in the aetiology of painful gastrointestinal disorder. J Psychosom Res 1984;28(5):411-21.

59. Ford MJ, Miller PM, Eastwood J, Eastwood MA. Life events, psychiatric illness and the irritable bowel syndrome. Gut 1987, Feb;28(2):160-5.

60. Toner BB, Garfinkel PE, Jeejeebhoy KN. Psychological factors in irritable bowel syndrome. Can J Psychiatry 1990, Mar;35(2):158-61.

61. Drossman DA, McKee DC, Sandler RS, Mitchell CM, Cramer EM, Lowman BC, Burger AL. Psychosocial factors in the irritable bowel syndrome. A multivariate study of patients and nonpatients with irritable bowel syndrome. Gastroenterology 1988, Sep;95(3):701-8.

62. Sandler RS, Drossman DA, Nathan HP, McKee DC. Symptom complaints and health care seeking behavior in subjects with bowel dysfunction. Gastroenterology 1984, Aug;87(2):314-8.

63. Kettell J, Jones R, Lydeard S. Reasons for consultation in irritable bowel syndrome: Symptoms and patient characteristics. Br J Gen Pract 1992, Nov;42(364):459-61.

64. Creed F, Craig T, Farmer R. Functional abdominal pain, psychiatric illness, and life events. Gut 1988;29(2):235-42.

65. Whitehead WE, Crowell MD, Robinson JC, Heller BR, Schuster MM. Effects of stressful life events on bowel symptoms: Subjects with irritable bowel syndrome compared with subjects without bowel dysfunction. Gut 1992, Jun;33(6):825-30.

66. Bennett EJ, Tennant CC, Piesse C, Badcock CA, Kellow JE. Level of chronic life stress predicts clinical outcome in irritable bowel syndrome. Gut 1998, Aug;43(2):256-61.

67. Drossman DA, Leserman J, Nachman G, Li ZM, Gluck H, Toomey TC, Mitchell CM. Sexual and physical abuse in women with functional or organic gastrointestinal disorders. Ann Intern Med 1990, Dec 1;113(11):828-33.

68. Talley NJ, Fett SL, Zinsmeister AR, Melton LJ. Gastrointestinal tract symptoms and self-reported abuse: A population-based study. Gastroenterology 1994, Oct;107(4):1040-9.

69. Talley NJ, Fett SL, Zinsmeister AR. Self-Reported abuse and gastrointestinal disease in outpatients: Association with irritable bowel-type symptoms. Am J Gastroenterol 1995, Mar;90(3):366-71.

70. Leroi AM, Bernier C, Watier A, Hémond M, Goupil G, Black R, et al. Prevalence of sexual abuse among patients with functional disorders of the lower gastrointestinal tract. Int J Colorectal Dis 1995;10(4):200-6.

71. Gwee KA, Graham JC, McKendrick MW, Collins SM, Marshall JS, Walters SJ, Read NW. Psychometric scores and persistence of irritable bowel after infectious diarrhoea. Lancet 1996, Jan 20;347(8995):150-3.

72. Neal KR, Barker L, Spiller RC. Prognosis in post-infective irritable bowel syndrome: A six year follow up study. Gut 2002, Sep;51(3):410-3.

73. Whitehead WE, Bosmajian L, Zonderman AB, Costa PT, Schuster MM. Symptoms of psychologic distress associated with irritable bowel syndrome. Comparison of community and medical clinic samples. Gastroenterology 1988, Sep;95(3):709-14.

74. Abell TL, Cutts TF, Cooper T. Effect of cisapride therapy for severe dyspepsia on gastrointestinal symptoms and quality of life. Scand J Gastroenterol Suppl 1993;195:60-3; discussion 63-4.

75. Talley NJ, Boyce PM, Jones M. Is the association between irritable bowel syndrome and abuse explained by neuroticism? A population based study. Gut 1998, Jan;42(1):47-53.

76. Chang L. Review article: Epidemiology and quality of life in functional gastrointestinal disorders. Aliment Pharmacol Ther 2004, Nov;20 Suppl 7:31-9.

77. Paganelli R, Quinti I, D'Offizi GP, Papetti C, Carini C, Aiuti F. Immune complexes in food allergy: A critical reappraisal. Annals of Allergy 1987;59(5 Pt 2):157.

78. Chandra RK. Food hypersensitivity and allergic disease: A selective review. Am J Clin Nutr 1997, Aug;66(2):526S-9S.

79. Burr ML, Merrett TG. Food intolerance: A community survey. British Journal of Nutrition 1983;49(02):217-.

80. Farup PG, Monsbakken KW, Vandvik PO. Lactose malabsorption in a population with irritable bowel syndrome: Prevalence and symptoms. A case-control study. Scand J Gastroenterol 2004, Jul;39(7):645-9.

81. Turnbull GK. Lactose intolerance and irritable bowel syndrome. Nutrition 2000;16(7-8):665-6.

82. Sciarretta G, Giacobazzi G, Verri A, Zanirato P, Garuti G, Malaguti P. Hydrogen breath test quantification and clinical correlation of lactose malabsorption in adult irritable bowel syndrome and ulcerative colitis. Dig Dis Sci 1984, Dec;29(12):1098-104.

83. Parker TJ, Woolner JT, Prevost AT, Tuffnell Q, Shorthouse M, Hunter JO. Irritable bowel syndrome: Is the search for lactose intolerance justified? Eur J Gastroenterol Hepatol 2001, Mar;13(3):219-25.

84. CHAUDHARY NA, TRUELOVE SC. The irritable colon syndrome. A study of the clinical features, predisposing causes, and prognosis in 130 cases. Q J Med 1962, Jul;31:307-22.

85. Isgar B, Harman M, Kaye MD, Whorwell PJ. Symptoms of irritable bowel syndrome in ulcerative colitis in remission. Gut 1983, Mar;24(3):190-2.

86. Minderhoud IM, Oldenburg B, Wismeijer JA, van Berge Henegouwen GP, Smout AJ. Ibs-Like symptoms in patients with inflammatory bowel disease in remission; relationships with quality of life and coping behavior. Dig Dis Sci 2004, Mar;49(3):469-74.

87. Simrén M, Axelsson J, Gillberg R, Abrahamsson H, Svedlund J, Björnsson ES. Quality of life in inflammatory bowel disease in remission: The impact of ibs-like symptoms and associated psychological factors. Am J Gastroenterol 2002, Feb;97(2):389-96.

88. Collins SM, Piche T, Rampal P. The putative role of inflammation in the irritable bowel syndrome. Gut 2001, Dec;49(6):743-5.

89. Loening-Baucke V, Metcalf AM, Shirazi S. Rectosigmoid motility in patients with quiescent and active ulcerative colitis. Am J Gastroenterol 1989, Jan;84(1):34-9.

90. Thaysen EH, Pedersen L. Idiopathic bile acid catharsis. Gut 1976, Dec;17(12):965-70.

91. Smith MJ, Cherian P, Raju GS, Dawson BF, Mahon S, Bardhan KD. Bile acid malabsorption in persistent diarrhoea. J R Coll Physicians Lond 2000;34(5):448-51.

92. Merrick MV, Eastwood MA, Ford MJ. Is bile acid malabsorption underdiagnosed? An evaluation of accuracy of diagnosis by measurement of sehcat retention. Br Med J (Clin Res Ed) 1985, Mar 2;290(6469):665-8.

93. Sinha L, Liston R, Testa HJ, Moriarty KJ. Idiopathic bile acid malabsorption: Qualitative and quantitative clinical features and response to cholestyramine. Aliment Pharmacol Ther 1998, Sep;12(9):839-44.

94. Cash BD, Schoenfeld P, Chey WD. The utility of diagnostic tests in irritable bowel syndrome patients: A systematic review. Am J Gastroenterol 2002, Nov;97(11):2812-9.

95. NSC NCC. Irritable bowel syndrome: NICE guideline. .

96. Longstreth GF, Drossman DA. Severe irritable bowel and functional abdominal pain syndromes: Managing the patient and health care costs. Clin Gastroenterol Hepatol 2005, Apr;3(4):397-400.

97. O'Sullivan MA, Mahmud N, Kelleher DP, Lovett E, O'Morain CA. Patient knowledge and educational needs in irritable bowel syndrome. Eur J Gastroenterol Hepatol 2000, Jan;12(1):39-43.

98. Owens DM, Nelson DK, Talley NJ. The irritable bowel syndrome: Long-Term prognosis and the physician-patient interaction. Ann Intern Med 1995, Jan 15;122(2):107-12.

99. Whitehead WE, Levy RL, Von Korff M, Feld AD, Palsson OS, Turner M, Drossman DA. The usual medical care for irritable bowel syndrome. Aliment Pharmacol Ther 2004, Dec;20(11-12):1305-15.

100. Thompson WG, Heaton KW, Smyth GT, Smyth C. Irritable bowel syndrome in general practice: Prevalence, characteristics, and referral. Gut 2000, Jan;46(1):78-82.

101. Heitkemper MM, Jarrett ME, Levy RL, Cain KC, Burr RL, Feld A, et al. Self-Management for women with irritable bowel syndrome. Clin Gastroenterol Hepatol 2004, Jul;2(7):585-96.

102. Saito YA, Prather CM, Van Dyke CT, Fett S, Zinsmeister AR, Locke GR. Effects of multidisciplinary education on outcomes in patients with irritable bowel syndrome. Clin Gastroenterol Hepatol 2004, Jul;2(7):576-84.

103. Harvey RF, Pomare EW, Heaton KW. Effects of increased dietary fibre on intestinal transit. Lancet 1973, Jun 9;1(7815):1278-80.

104. Francis CY, Whorwell PJ. Bran and irritable bowel syndrome: Time for reappraisal. Lancet 1994, Jul 2;344(8914):39-40.

105. Lewis MJ, Whorwell PJ. Bran: May irritate irritable bowel. Nutrition 1998, May;14(5):470-1.

106. Suarez FL, Savaiano DA, Levitt MD. A comparison of symptoms after the consumption of milk or lactose-hydrolyzed milk by people with self-reported severe lactose intolerance. N Engl J Med 1995, Jul 6;333(1):1-4.

107. Attar A, Lémann M, Ferguson A, Halphen M, Boutron MC, Flourié B, et al. Comparison of a low dose polyethylene glycol electrolyte solution with lactulose for treatment of chronic constipation. Gut 1999, Feb;44(2):226-30.

108. Davis K, Philpott S, Kumar D, Mendall M. Randomised double-blind placebo-controlled trial of aloe vera for irritable bowel syndrome. Int J Clin Pract 2006, Sep;60(9):1080-6.

109. van Tilburg MA, Palsson OS, Levy RL, Feld AD, Turner MJ, Drossman DA, Whitehead WE. Complementary and alternative medicine use and cost in functional bowel disorders: A six month prospective study in a large HMO. BMC Complement Altern Med 2008, Jul 24;8(1):46.

110. Schneider A, Enck P, Streitberger K, Weiland C, Bagheri S, Witte S, et al. Acupuncture treatment in irritable bowel syndrome. Gut 2006, May;55(5):649-54.

111. Tovey P. A single-blind trial of reflexology for irritable bowel syndrome. Br J Gen Pract 2002, Jan;52(474):19-23.

112. Amery W, Duyck F, Polak J, van den Bouwhuysen G. A multicentre double-blind study in acute diarrhoea comparing loperamide (R 18553) with two common antidiarrhoeal agents and a placebo. Curr Ther Res Clin Exp 1975, Mar;17(3):263-70.

113. Harford WV, Krejs GJ, Santa Ana CA, Fordtran JS. Acute effect of diphenoxylate with atropine (lomotil) in patients with chronic diarrhea and fecal incontinence. Gastroenterology 1980, Mar;78(3):440-3.

114. Lustman F, Walters EG, Shroff NE, Akbar FA. Diphenoxylate hydrochloride (lomotil) in the treatment of acute diarrhoea. Br J Clin Pract 1987, Mar;41(3):648-51.

115. Quartero AO, Meineche-Schmidt V, Muris J, Rubin G, de Wit N. Bulking agents, antispasmodic and antidepressant medication for the treatment of irritable bowel syndrome. Cochrane Database Syst Rev 2005(2):CD003460.

116. Poynard T, Naveau S, Mory B, Chaput JC. Meta-Analysis of smooth muscle relaxants in the treatment of irritable bowel syndrome. Aliment Pharmacol Ther 1994, Oct;8(5):499-510.

117. Klein KB. Controlled treatment trials in the irritable bowel syndrome: A critique. Gastroenterology 1988, Jul;95(1):232-41.

118. Jailwala J, Imperiale TF, Kroenke K. Pharmacologic treatment of the irritable bowel syndrome: A systematic review of randomized, controlled trials. Ann Intern Med 2000, Jul 18;133(2):136-47.

119. Poynard T, Regimbeau C, Benhamou Y. Meta-Analysis of smooth muscle relaxants in the treatment of irritable bowel syndrome. Aliment Pharmacol Ther 2001, Mar;15(3):355-61.

120. Drossman DA, Toner BB, Whitehead WE, Diamant NE, Dalton CB, Duncan S, et al. Cognitive-Behavioral therapy versus education and desipramine versus placebo for moderate to severe functional bowel disorders. Gastroenterology 2003, Jul;125(1):19-31.

121. Fioramonti J, Bueno L. Centrally acting agents and visceral sensitivity. Gut 2002, Jul;51 Suppl 1:i91-5.

122. Drossman DA, Camilleri M, Mayer EA, Whitehead WE. AGA technical review on irritable bowel syndrome. Gastroenterology 2002, Dec;123(6):2108-31.

123. Jackson JL, O'Malley PG, Tomkins G, Balden E, Santoro J, Kroenke K. Treatment of functional gastrointestinal disorders with antidepressant medications: A meta-analysis. Am J Med 2000, Jan;108(1):65-72.

124. Creed F, Fernandes L, Guthrie E, Palmer S, Ratcliffe J, Read N, et al. The cost-effectiveness of psychotherapy and paroxetine for severe irritable bowel syndrome. Gastroenterology 2003, Feb;124(2):303-17.

125. Kuiken SD, Tytgat GN, Boeckxstaens GE. The selective serotonin reuptake inhibitor fluoxetine does not change rectal sensitivity and symptoms in patients with irritable bowel syndrome: A double blind, randomized, placebo-controlled study. Clin Gastroenterol Hepatol 2003, May;1(3):219-28.

126. Tabas G, Beaves M, Wang J, Friday P, Mardini H, Arnold G. Paroxetine to treat irritable bowel syndrome not responding to high-fiber diet: A double-blind, placebo-controlled trial. Am J Gastroenterol 2004, May;99(5):914-20.

127. Tack J, Broekaert D, Fischler B, Van Oudenhove L, Gevers AM, Janssens J. A controlled crossover study of the selective serotonin reuptake inhibitor citalopram in irritable bowel syndrome. Gut 2006, Aug;55(8):1095-103.

128. Robinson A, Lee V, Kennedy A, Middleton L, Rogers A, Thompson DG, Reeves D. A randomised controlled trial of self-help interventions in patients with a primary care diagnosis of irritable bowel syndrome. Gut 2006, May;55(5):643-8.

129. Lacy BE, Weiser K, Noddin L, Robertson DJ, Crowell MD, Parratt-Engstrom C, Grau MV. Irritable bowel syndrome: Patients' attitudes, concerns and level of knowledge. Aliment Pharmacol Ther 2007, Jun 1;25(11):1329-41.

130. Kennedy A, Robinson A, Rogers A. Incorporating patients' views and experiences of life with IBS in the development of an evidence based self-help guidebook. Patient Educ Couns 2003, Jul;50(3):303-10.

131. Kennedy A, Rogers A, Bower P. Support for self care for patients with chronic disease. BMJ 2007, Nov 10;335(7627):968-70.

132. Forbes A, MacAuley S, Chiotakakou-Faliakou E. Hypnotherapy and therapeutic audiotape: Effective in previously unsuccessfully treated irritable bowel syndrome? Int J Colorectal Dis 2000, Nov;15(5-6):328-34.

133. Tan G, Hammond DC, Joseph G. Hypnosis and irritable bowel syndrome: A review of efficacy and mechanism of action. Am J Clin Hypn 2005, Jan;47(3):161-78.

134. Palsson OS, Whitehead WE. The growing case for hypnosis as adjunctive therapy for functional gastrointestinal disorders. Gastroenterology 2002, Dec;123(6):2132-5.

135. Palsson OS, Turner MJ, Whitehead WE. Hypnosis home treatment for irritable bowel syndrome: A pilot study. Int J Clin Exp Hypn 2006, Jan;54(1):85-99.

136. Gonsalkorale WM, Miller V, Afzal A, Whorwell PJ. Long term benefits of hypnotherapy for irritable bowel syndrome. Gut 2003, Nov;52(11):1623-9.

137. Prior A, Colgan SM, Whorwell PJ. Changes in rectal sensitivity after hypnotherapy in patients with irritable bowel syndrome. Gut 1990, Aug;31(8):896-8.

138. Lea R, Houghton LA, Calvert EL, Larder S, Gonsalkorale WM, Whelan V, et al. Gut-Focused hypnotherapy normalizes disordered rectal sensitivity in patients with irritable bowel syndrome. Aliment Pharmacol Ther 2003, Mar 1;17(5):635-42.

139. Gonsalkorale WM, Toner BB, Whorwell PJ. Cognitive change in patients undergoing hypnotherapy for irritable bowel syndrome. J Psychosom Res 2004, Mar;56(3):271-8.

140. Gonsalkorale WM, Houghton LA, Whorwell PJ. Hypnotherapy in irritable bowel syndrome: A large-scale audit of a clinical service with examination of factors influencing responsiveness. Am J Gastroenterol 2002, Apr;97(4):954-61.

141. Toner BB, Segal ZV, Emmott S, Myran D, Ali A, DiGasbarro I, Stuckless N. Cognitive-Behavioral group therapy for patients with irritable bowel syndrome. Int J Group Psychother 1998, Apr;48(2):215-43.

142. Levy RL, Olden KW, Naliboff BD, Bradley LA, Francisconi C, Drossman DA, Creed F. Psychosocial aspects of the functional gastrointestinal disorders. Gastroenterology 2006, Apr;130(5):1447-58.

143. Bennett P, Wilkinson S. A comparison of psychological and medical treatment of the irritable bowel syndrome. Br J Clin Psychol 1985, Sep;24 (Pt 3):215-6.

144. Greene B, Blanchard EB. Cognitive therapy for irritable bowel syndrome. J Consult Clin Psychol 1994, Jun;62(3):576-82.

145. Payne A, Blanchard EB. A controlled comparison of cognitive therapy and self-help support groups in the treatment of irritable bowel syndrome. J Consult Clin Psychol 1995, Oct;63(5):779-86.

146. Svedlund J, Sjödin I, Ottosson JO, Dotevall G. Controlled study of psychotherapy in irritable bowel syndrome. Lancet 1983, Sep 10;2(8350):589-92.

147. Guthrie E, Creed F, Dawson D, Tomenson B. A controlled trial of psychological treatment for the irritable bowel syndrome. Gastroenterology 1991, Feb;100(2):450-7.

148. Guthrie E, Barlow J, Fernandes L, Ratcliffe J, Read N, Thompson DG, et al. Changes in tolerance to rectal distension correlate with changes in psychological state in patients with severe irritable bowel syndrome. Psychosom Med 2004;66(4):578-82.

149. Creed F, Guthrie E, Ratcliffe J, Fernandes L, Rigby C, Tomenson B, et al. Reported sexual abuse predicts impaired functioning but a good response to psychological treatments in patients with severe irritable bowel syndrome. Psychosom Med 2005;67(3):490-9.

150. Pilowsky I. Abnormal illness behaviour. Br J Med Psychol 1969, Dec;42(4):347-51.

151. Pilowsky I. The concept of abnormal illness behavior. Psychosomatics 1990;31(2):207-13.

152. MECHANIC D. The concept of illness behavior. J Chronic Dis 1962, Feb;15:189-94.

153. PARSONS T. Illness and the role of the physician: A sociological perspective. Am J Orthopsychiatry 1951, Jul;21(3):452-60.

154. Levy RL, Whitehead WE, Von Korff MR, Feld AD. Intergenerational transmission of gastrointestinal illness behavior. Am J Gastroenterol 2000, Feb;95(2):451-6.

155. Lowman BC, Drossman DA, Cramer EM, McKee DC. Recollection of childhood events in adults with irritable bowel syndrome. J Clin Gastroenterol 1987, Jun;9(3):324-30.

156. Whitehead WE, Winget C, Fedoravicius AS, Wooley S, Blackwell B. Learned illness behavior in patients with irritable bowel syndrome and peptic ulcer. Dig Dis Sci 1982;27(3):202-8.

157. Whitehead WE, Crowell MD, Heller BR, Robinson JC, Schuster MM, Horn S. Modeling and reinforcement of the sick role during childhood predicts adult illness behavior. Psychosom Med 1994;56(6):541-50.

158. Hill OW, Blendis L. Physical and psychological evaluation of 'non-organic' abdominal pain. Gut 1967, Jun;8(3):221-9.

159. Schuster MM, Whitehead WE. Physiologic insights into irritable bowel syndrome. Clin Gastroenterol 1986, Oct;15(4):839-53.

160. Koloski NA, Boyce PM, Talley NJ. Is health care seeking for irritable bowel syndrome and functional dyspepsia a socially learned response to illness? Dig Dis Sci 2005;50(1):153-62.

161. Talley NJ, Boyce PM, Jones M. Predictors of health care seeking for irritable bowel syndrome: A population based study. Gut 1997, Sep;41(3):394-8.

162. Howell S, Poulton R, Talley NJ. The natural history of childhood abdominal pain and its association with adult irritable bowel syndrome: Birth-Cohort study. Am J Gastroenterol 2005, Sep;100(9):2071-8.

163. Gick ML, Thompson WG. Negative affect and the seeking of medical care in university students with irritable bowel syndrome: A preliminary study. J Psychosom Res 1997, Nov;43(5):535-40.

164. Howell S, Talley NJ. Does fear of serious disease predict consulting behaviour amongst patients with dyspepsia in general practice? Eur J Gastroenterol Hepatol 1999, Aug;11(8):881-6.

165. Engel GL. The need for a new medical model: A challenge for biomedicine. Science 1977, Apr 8;196(4286):129-36.

166. Longstreth GF, Hawkey CJ, Mayer EA, Jones RH, Naesdal J, Wilson IK, et al. Characteristics of patients with irritable bowel syndrome recruited from three sources: Implications for clinical trials. Aliment Pharmacol Ther 2001, Jul;15(7):959-64.

167. Rodríguez LA, Ruigómez A. Increased risk of irritable bowel syndrome after bacterial gastroenteritis: Cohort study. BMJ 1999, Feb 27;318(7183):565-6.

168. Spiller RC. Postinfectious irritable bowel syndrome. Gastroenterology 2003, May;124(6):1662-71.

169. Thabane M, Kottachchi DT, Marshall JK. Systematic review and meta-analysis: The incidence and prognosis of post-infectious irritable bowel syndrome. Aliment Pharmacol Ther 2007, Aug 15;26(4):535-44.

170. STEWART GT. Post-Dysenteric colitis. Br Med J 1950, Feb 18;1(4650):405-9.

171. Okhuysen PC, Jiang ZD, Carlin L, Forbes C, DuPont HL. Post-Diarrhea chronic intestinal symptoms and irritable bowel syndrome in north american travelers to mexico. Am J Gastroenterol 2004, Sep;99(9):1774-8.

172. Parry SD, Stansfield R, Jelley D, Gregory W, Phillips E, Barton JR, Welfare MR. Does bacterial gastroenteritis predispose people to functional gastrointestinal disorders? A prospective, community-based, case-control study. Am J Gastroenterol 2003, Sep;98(9):1970-5.

173. McKendrick MW, Read NW. Irritable bowel syndrome--post salmonella infection. J Infect 1994, Jul;29(1):1-3.

174. Mearin F, Pérez-Oliveras M, Perelló A, Vinyet J, Ibañez A, Coderch J, Perona M. Dyspepsia and irritable bowel syndrome after a salmonella gastroenteritis outbreak: One-Year follow-up cohort study. Gastroenterology 2005, Jul;129(1):98-104.

175. Ji S, Park H, Lee D, Song YK, Choi JP, Lee SI. Post-Infectious irritable bowel syndrome in patients with shigella infection. J Gastroenterol Hepatol 2005, Mar;20(3):381-6.

176. Kim HS, Kim MS, Ji SW, Park H. [The development of irritable bowel syndrome after shigella infection: 3 year follow-up study]. Korean J Gastroenterol 2006, Apr;47(4):300-5.

177. Soyturk M, Akpinar H, Gurler O, Pozio E, Sari I, Akar S, et al. Irritable bowel syndrome in persons who acquired trichinellosis. Am J Gastroenterol 2007, May;102(5):1064-9.

178. Marshall JK, Thabane M, Garg AX, Clark WF, Salvadori M, Collins SM, Walkerton Health Study Investigators. Incidence and epidemiology of irritable bowel syndrome after a large waterborne outbreak of bacterial dysentery. Gastroenterology 2006, Aug;131(2):445-50; quiz 660.

179. Marshall JK, Thabane M, Borgaonkar MR, James C. Postinfectious irritable bowel syndrome after a food-borne outbreak of acute gastroenteritis attributed to a viral pathogen. Clin Gastroenterol Hepatol 2007, Apr;5(4):457-60.

180. Halvorson HA, Schlett CD, Riddle MS. Postinfectious irritable bowel syndrome--a meta-analysis. Am J Gastroenterol 2006, Aug;101(8):1894-9; quiz 1942.

181. Spence MJ, Moss-Morris R. The cognitive behavioural model of irritable bowel syndrome: A prospective investigation of patients with gastroenteritis. Gut 2007, Aug;56(8):1066-71.

182. Dunlop SP, Jenkins D, Neal KR, Spiller RC. Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. Gastroenterology 2003, Dec;125(6):1651-9.

183. Kristjánsson G, Venge P, Wanders A, Lööf L, Hällgren R. Clinical and subclinical intestinal inflammation assessed by the mucosal patch technique: Studies of mucosal neutrophil and eosinophil activation in inflammatory bowel diseases and irritable bowel syndrome. Gut 2004, Dec;53(12):1806-12.

184. Schoepfer AM, Trummler M, Seeholzer P, Seibold-Schmid B, Seibold F. Discriminating IBD from IBS: Comparison of the test performance of fecal markers, blood leukocytes, CRP, and IBD antibodies. Inflamm Bowel Dis 2008, Jan;14(1):32-9.

185. Wheatcroft J, Wakelin D, Smith A, Mahoney CR, Mawe G, Spiller R. Enterochromaffin cell hyperplasia and decreased serotonin transporter in a mouse model of postinfectious bowel dysfunction. Neurogastroenterol Motil 2005, Dec;17(6):863-70.

186. Wang LH, Fang XC, Pan GZ. Bacillary dysentery as a causative factor of irritable bowel syndrome and its pathogenesis. Gut 2004, Aug;53(8):1096-101.

187. Gwee KA, Collins SM, Read NW, Rajnakova A, Deng Y, Graham JC, et al. Increased rectal mucosal expression of interleukin 1beta in recently acquired post-infectious irritable bowel syndrome. Gut 2003, Apr;52(4):523-6.

188. Chadwick VS, Chen W, Shu D, Paulus B, Bethwaite P, Tie A, Wilson I. Activation of the mucosal immune system in irritable bowel syndrome. Gastroenterology 2002, Jun;122(7):1778-83.

189. Gonsalkorale WM, Perrey C, Pravica V, Whorwell PJ, Hutchinson IV. Interleukin 10 genotypes in irritable bowel syndrome: Evidence for an inflammatory component? Gut 2003, Jan;52(1):91-3.

190. Spiller RC, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner M, Neal KR. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute campylobacter enteritis and in post-dysenteric irritable bowel syndrome. Gut 2000, Dec;47(6):804-11.

191. Söderholm JD, Yates DA, Gareau MG, Yang PC, MacQueen G, Perdue MH. Neonatal maternal separation predisposes adult rats to colonic barrier dysfunction in response to mild stress. Am J Physiol Gastrointest Liver Physiol 2002, Dec;283(6):G1257-63.

192. Zareie M, Johnson-Henry K, Jury J, Yang PC, Ngan BY, McKay DM, et al. Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. Gut 2006, Nov;55(11):1553-60.

193. Yang PC, Jury J, Söderholm JD, Sherman PM, McKay DM, Perdue MH. Chronic psychological stress in rats induces intestinal sensitization to luminal antigens. Am J Pathol 2006, Jan;168(1):104-14; quiz 363.

194. Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, et al. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterology 2004, Mar;126(3):693-702.

195. Farthing MJ, Lennard-jones JE. Sensibility of the rectum to distension and the anorectal distension reflex in ulcerative colitis. Gut 1978, Jan;19(1):64-9.

196. Rao SS, Read NW, Davison PA, Bannister JJ, Holdsworth CD. Anorectal sensitivity and responses to rectal distention in patients with ulcerative colitis. Gastroenterology 1987, Dec;93(6):1270-5.

197. Chang L, Munakata J, Mayer EA, Schmulson MJ, Johnson TD, Bernstein CN, et al. Perceptual responses in patients with inflammatory and functional bowel disease. Gut 2000, Oct;47(4):497-505.

198. Meddings JB. Review article: Intestinal permeability in crohn's disease. Aliment Pharmacol Ther 1997, Dec;11 Suppl 3:47-53; discussion 53-6.

199. Marshall JK, Thabane M, Garg AX, Clark W, Meddings J, Collins SM, WEL Investigators. Intestinal permeability in patients with irritable bowel syndrome after a waterborne outbreak of acute gastroenteritis in walkerton, ontario. Aliment Pharmacol Ther 2004, Dec;20(11-12):1317-22.

200. Dunlop SP, Hebden J, Campbell E, Naesdal J, Olbe L, Perkins AC, Spiller RC. Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. Am J Gastroenterol 2006, Jun;101(6):1288-94.

201. Berkes J, Viswanathan VK, Savkovic SD, Hecht G. Intestinal epithelial responses to enteric pathogens: Effects on the tight junction barrier, ion transport, and inflammation. Gut 2003, Mar;52(3):439-51.

202. D'Incà R, Di Leo V, Corrao G, Martines D, D'Odorico A, Mestriner C, et al. Intestinal permeability test as a predictor of clinical course in crohn's disease. Am J Gastroenterol 1999, Oct;94(10):2956-60.

203. Hollander D, Vadheim CM, Brettholz E, Petersen GM, Delahunty T, Rotter JI. Increased intestinal permeability in patients with crohn's disease and their relatives. A possible etiologic factor. Ann Intern Med 1986, Dec;105(6):883-5.

204. Bjarnason I, Marsh MN, Price A, Levi AJ, Peters TJ. Intestinal permeability in patients with coeliac disease and dermatitis herpetiformis. Gut 1985, Nov;26(11):1214-9.

205. Clayburgh DR, Barrett TA, Tang Y, Meddings JB, Van Eldik LJ, Watterson DM, et al. Epithelial myosin light chain kinase-dependent barrier dysfunction mediates T cell activation-induced diarrhea in vivo. J Clin Invest 2005, Oct;115(10):2702-15.

206. Blair SA, Kane SV, Clayburgh DR, Turner JR. Epithelial myosin light chain kinase expression and activity are upregulated in inflammatory bowel disease. Lab Invest 2006, Feb;86(2):191-201.

207. Zolotarevsky Y, Hecht G, Koutsouris A, Gonzalez DE, Quan C, Tom J, et al. A membrane-permeant peptide that inhibits MLC kinase restores barrier function in in vitro models of intestinal disease. Gastroenterology 2002, Jul;123(1):163-72.

208. Whitehead WE, Holtkotter B, Enck P, Hoelzl R, Holmes KD, Anthony J, et al. Tolerance for rectosigmoid distention in irritable bowel syndrome. Gastroenterology 1990, May;98(5 Pt 1):1187-92.

209. Hobson AR, Furlong PL, Sarkar S, Matthews PJ, Willert RP, Worthen SF, et al. Neurophysiologic assessment of esophageal sensory processing in noncardiac chest pain. Gastroenterology 2006, Jan;130(1):80-8.

210. Harris ML, Hobson AR, Hamdy S, Thompson DG, Akkermans LM, Aziz Q. Neurophysiological evaluation of healthy human anorectal sensation. Am J Physiol Gastrointest Liver Physiol 2006, Nov;291(5):G950-8.

211. Murray CD, Flynn J, Ratcliffe L, Jacyna MR, Kamm MA, Emmanuel AV. Effect of acute physical and psychological stress on gut autonomic innervation in irritable bowel syndrome. Gastroenterology 2004, Dec;127(6):1695-703.

212. Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: A simple method of monitoring irritable bowel syndrome and its progress. Aliment Pharmacol Ther 1997, Apr;11(2):395-402.

213. Drossman DA, Li Z, Toner BB, Diamant NE, Creed FH, Thompson D, et al. Functional bowel disorders. A multicenter comparison of health status and development of illness severity index. Dig Dis Sci 1995, May;40(5):986-95.

214. Gracely RH, McGrath P, Dubner R. Validity and sensitivity of ratio scales of sensory and affective verbal pain descriptors: Manipulation of affect by diazepam. Pain 1978, Jun;5(1):19-29.

215. Huskisson EC. Measurement of pain. J Rheumatol 1982;9(5):768-9.

216. Guyatt GH, Townsend M, Berman LB, Keller JL. A comparison of likert and visual analogue scales for measuring change in function. J Chronic Dis 1987;40(12):1129-33.

217. Likert R. A technique for the measurement of attitudes. 1932.

218. Huskisson EC. Measurement of pain. Lancet 1974, Nov 9;2(7889):1127-31.

219. Sriwatanakul K, Kelvie W, Lasagna L, Calimlim JF, Weis OF, Mehta G. Studies with different types of visual analog scales for measurement of pain. Clin Pharmacol Ther 1983, Aug;34(2):234-9.

220. Naliboff BD, Fullerton S, Mayer EA. Measurement of symptoms in irritable bowel syndrome clinical trials. Am J Med 1999, Nov 8;107(5A):81S-4S.

221. McDowell I. Measuring health: A guide to rating scales and questionnaires. Oxford University Press.

222. Houghton LA, Heyman DJ, Whorwell PJ. Symptomatology, quality of life and economic features of irritable bowel syndrome--the effect of hypnotherapy. Aliment Pharmacol Ther 1996, Feb;10(1):91-5.

223. Farup PG, Hovdenak N, Wetterhus S, Lange OJ, Hovde O, Trondstad R. The symptomatic effect of cisapride in patients with irritable bowel syndrome and constipation. Scand J Gastroenterol 1998, Feb;33(2):128-31.

224. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983, Jun;67(6):361-70.

225. Toner BB, Garfinkel PE, Jeejeebhoy KN. Psychological factors in irritable bowel syndrome. Can J Psychiatry 1990, Mar;35(2):158-61.

226. Dinan TG, O'Keane V, O'Boyle C, Chua A, Keeling PW. A comparison of the mental status, personality profiles and life events of patients with irritable bowel syndrome and peptic ulcer disease. Acta Psychiatr Scand 1991, Jul;84(1):26-8.

227. Fock KM, Chew CN, Tay LK, Peh LH, Chan S, Pang EP. Psychiatric illness, personality traits and the irritable bowel syndrome. Ann Acad Med Singapore 2001, Nov;30(6):611-4.

228. Talley NJ, Boyce P, Owen BK. Psychological distress and seasonal symptom changes in irritable bowel syndrome. Am J Gastroenterol 1995, Dec;90(12):2115-9.

229. Talley NJ, Boyce PM, Jones M. Predictors of health care seeking for irritable bowel syndrome: A population based study. Gut 1997, Sep;41(3):394-8.

230. Pilowsky I. Dimensions of hypochondriasis. Br J Psychiatry 1967, Jan;113(494):89-93.

231. Spitzer RL, Kroenke K, Williams JB. Validation and utility of a self-report version of PRIME-MD: The PHQ primary care study. Primary care evaluation of mental disorders. Patient health questionnaire. JAMA 1999, Nov 10;282(18):1737-44.

232. Spitzer RL, Williams JB, Kroenke K, Linzer M, deGruy FV, Hahn SR, et al. Utility of a new procedure for diagnosing mental disorders in primary care. The PRIME-MD 1000 study. JAMA 1994, Dec 14;272(22):1749-56.

233. Kroenke K, Spitzer RL, Williams JB. The PHQ-15: Validity of a new measure for evaluating the severity of somatic symptoms. Psychosom Med 2002;64(2):258-66.

234. Mayer EA, Naliboff BD, Chang L, Coutinho SV. V. Stress and irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol 2001, Apr;280(4):G519-24.

235. Reiss S. Trait anxiety: It's not what you think it is. J Anxiety Disord 1997;11(2):201-14.

236. Spielberger CD. Anxiety: State-Trait process.. In: Stress and anxiety. Washington, DC: Hemisphere. ; 1975.

237. Mayer EA, Craske M, Naliboff BD. Depression, anxiety, and the gastrointestinal system. J Clin Psychiatry 2001;62 Suppl 8:28-36; discussion 37.

238. Cattell RB, Scheier IH. The meaning and measurement of neuroticism and anxiety. Ronald; 1961.

239. Freud S. A general introduction to psychoanalysis. Boni and Liveright; 1920.

240. Spielberger CD, Gorsuch RL. STAI manual for the state-trait anxiety inventory (" self-evaluation questionnaire"). Consulting Psychologists Press; 1970.

241. Spielberger CD, Gorsuch RL, Lushene R, Vagg PR, Jacobs GA. State-Trait anxiety inventory for adults. Consulting Psychologists Press Palo Alto, CA; 1983.

242. Barnes LLB, Harp D, Jung WS. Reliability generalization of scores on the spielberger state-trait anxiety inventory. Educational and Psychological Measurement 2002;62(4):603.

243. Longstreth GF, Bolus R, Naliboff B, Chang L, Kulich KR, Carlsson J, et al. Impact of irritable bowel syndrome on patients' lives: Development and psychometric documentation of a disease-specific measure for use in clinical trials. Eur J Gastroenterol Hepatol 2005, Apr;17(4):411-20.

244. Patrick DL, Drossman DA, Frederick IO, DiCesare J, Puder KL. Quality of life in persons with irritable bowel syndrome: Development and validation of a new measure. Dig Dis Sci 1998, Feb;43(2):400-11.

245. Chassany O, Marquis P, Scherrer B, Read NW, Finger T, Bergmann JF, et al. Validation of a specific quality of life questionnaire for functional digestive disorders. Gut 1999, Apr;44(4):527-33.

246. Wong E, Guyatt GH, Cook DJ, Griffith LE, Irvine EJ. Development of a questionnaire to measure quality of life in patients with irritable bowel syndrome. Eur J Surg Suppl 1998(583):50-6.

247. Hahn BA, Kirchdoerfer LJ, Fullerton S, Mayer E. Evaluation of a new quality of life questionnaire for patients with irritable bowel syndrome. Aliment Pharmacol Ther 1997, Jun;11(3):547-52.

248. Ghani AC, Donnelly CA, Garnett GP. Sampling biases and missing data in explorations of sexual partner networks for the spread of sexually transmitted diseases. Stat Med 1998, Sep 30;17(18):2079-97.

249. Wyrwich KW, Tardino VM. A blueprint for symptom scales and responses: Measurement and reporting. Gut 2004, May;53 Suppl 4:iv45-8.

250. Meunier P, Collet L, Duclaux R, Chery-Croze S. Endorectal cerebral evoked potentials in human. Int J Neurosci 1987, Dec;37(3-4):193-6.

251. Collet L, Meunier P, Duclaux R, Chery-Croze S, Falipou P. Cerebral evoked potentials after endorectal mechanical stimulation in humans. Am J Physiol 1988, Apr;254(4 Pt 1):G477-82.

252. Frieling T, Enck P, Wienbeck M. Cerebral responses evoked by electrical stimulation of rectosigmoid in normal subjects. Dig Dis Sci 1989, Feb;34(2):202-5.

253. Loening-Baucke V, Read NW, Yamada T. Cerebral evoked potentials after rectal stimulation. Electroencephalogr Clin Neurophysiol 1991;80(6):490-5.

254. Hollerbach S, Fitzpatrick D, Shine G, Kamath MV, Upton AR, Tougas G. Cognitive evoked potentials to anticipated oesophageal stimulus in humans: Quantitative assessment of the cognitive aspects of visceral perception. Neurogastroenterol Motil 1999, Feb;11(1):37-46.

255. Hobday DI, Hobson AR, Sarkar S, Furlong PL, Thompson DG, Aziz Q. Cortical processing of human gut sensation: An evoked potential study. Am J Physiol Gastrointest Liver Physiol 2002, Aug;283(2):G335-9.

256. Engel GL. The need for a new medical model: A challenge for biomedicine. Science 1977, Apr 8;196(4286):129-36.

257. Drossman DA, Creed FH, Olden KW, Svedlund J, Toner BB, Whitehead WE. Psychosocial aspects of the functional gastrointestinal disorders. Gut 1999, Sep;45 Suppl 2:II25-30.

258. MacDonald AJ, Bouchier IA. Non-Organic gastrointestinal illness: A medical and psychiatric study. Br J Psychiatry 1980, Mar;136:276-83.

259. Colgan S, Creed F, Klass H. Symptom complaints, psychiatric disorder and abnormal illness behaviour in patients with upper abdominal pain. Psychol Med 1988, Nov;18(4):887-92.

260. Corney RH, Stanton R. Physical symptom severity, psychological and social dysfunction in a series of outpatients with irritable bowel syndrome. J Psychosom Res 1990;34(5):483-91.

261. Craig TK, Brown GW. Goal frustration and life events in the aetiology of painful gastrointestinal disorder. J Psychosom Res 1984;28(5):411-21.

262. Ford MJ, Miller PM, Eastwood J, Eastwood MA. Life events, psychiatric illness and the irritable bowel syndrome. Gut 1987, Feb;28(2):160-5.

263. Toner BB, Garfinkel PE, Jeejeebhoy KN. Psychological factors in irritable bowel syndrome. Can J Psychiatry 1990, Mar;35(2):158-61.

264. Lydiard RB. Irritable bowel syndrome, anxiety, and depression: What are the links? J Clin Psychiatry 2001;62 Suppl 8:38-45; discussion 46-7.

265. Lydiard RB, Falsetti SA. Experience with anxiety and depression treatment studies: Implications for designing irritable bowel syndrome clinical trials. Am J Med 1999, Nov 8;107(5A):65S-73S.

266. van Hemert AM, Hengeveld MW, Bolk JH, Rooijmans HG, Vandenbroucke JP. Psychiatric disorders in relation to medical illness among patients of a general medical out-patient clinic. Psychol Med 1993, Feb;23(1):167-73.

267. Ansseau M, Dierick M, Buntinkx F, Cnockaert P, De Smedt J, Van Den Haute M, Vander Mijnsbrugge D. High prevalence of mental disorders in primary care. J Affect Disord 2004, Jan;78(1):49-55.

268. Blanchard EB, Lackner JM, Jaccard J, Rowell D, Carosella AM, Powell C, et al. The role of stress in symptom exacerbation among IBS patients. J Psychosom Res 2008, Feb;64(2):119-28.

269. Sterling P, Eyer J. Allostasis: A new paradigm to explain arousal pathology. Handbook of Life Stress, Cognition and Health 1988.

270. McEwen BS, Stellar E. Stress and the individual. Mechanisms leading to disease. Arch Intern Med 1993, Sep 27;153(18):2093-101.

271. McEwen BS. Stress, adaptation, and disease. Allostasis and allostatic load. Ann N Y Acad Sci 1998, May 1;840:33-44.

272. Felitti VJ, Anda RF, Nordenberg D, Williamson DF, Spitz AM, Edwards V, et al. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The adverse childhood experiences (ACE) study. Am J Prev Med 1998, May;14(4):245-58.

273. Spertus IL, Yehuda R, Wong CM, Halligan S, Seremetis SV. Childhood emotional abuse and neglect as predictors of psychological and physical symptoms in women presenting to a primary care practice. Child Abuse Negl 2003, Nov;27(11):1247-58.

274. Dube SR, Anda RF, Felitti VJ, Chapman DP, Williamson DF, Giles WH. Childhood abuse, household dysfunction, and the risk of attempted suicide throughout the life span: Findings from the adverse childhood experiences study. JAMA 2001, Dec 26;286(24):3089-96.

275. Danese A, Moffitt TE, Harrington H, Milne BJ, Polanczyk G, Pariante CM, et al. Adverse childhood experiences and adult risk factors for age-related disease: Depression, inflammation, and clustering of metabolic risk markers. Arch Pediatr Adolesc Med 2009, Dec;163(12):1135-43.

276. Anda RF, Felitti VJ, Bremner JD, Walker JD, Whitfield C, Perry BD, et al. The enduring effects of abuse and related adverse experiences in childhood. A convergence of evidence from neurobiology and epidemiology. Eur Arch Psychiatry Clin Neurosci 2006, Apr;256(3):174-86.

277. Chapman DP, Whitfield CL, Felitti VJ, Dube SR, Edwards VJ, Anda RF. Adverse childhood experiences and the risk of depressive disorders in adulthood. J Affect Disord 2004, Oct 15;82(2):217-25.

278. Drossman DA. Sexual and physical abuse and gastrointestinal illness. Scand J Gastroenterol Suppl 1995;208:90-6.

279. Chitkara DK, van Tilburg MA, Blois-Martin N, Whitehead WE. Early life risk factors that contribute to irritable bowel syndrome in adults: A systematic review. Am J Gastroenterol 2008, Mar;103(3):765-74; quiz 775.

280. Bennett EJ, Tennant CC, Piesse C, Badcock CA, Kellow JE. Level of chronic life stress predicts clinical outcome in irritable bowel syndrome. British Medical Journal 1998;43(2):256-61.

281. Gwee KA, Leong YL, Graham C, McKendrick MW, Collins SM, Walters SJ, et al. The role of psychological and biological factors in postinfective gut dysfunction. British Medical Journal 1999;44(3):400-6.

282. Jarrett M, Heitkemper M, Cain KC, Tuftin M, Walker EA, Bond EF, Levy RL. The relationship between psychological distress and gastrointestinal symptoms in women with irritable bowel syndrome. Nurs Res 1998;47(3):154-61.

283. Levy RL, Cain KC, Jarrett M, Heitkemper MM. The relationship between daily life stress and gastrointestinal symptoms in women with irritable bowel syndrome. J Behav Med 1997, Apr;20(2):177-93.

284. Hertig VL, Cain KC, Jarrett ME, Burr RL, Heitkemper MM. Daily stress and gastrointestinal symptoms in women with irritable bowel syndrome. Nurs Res 2007;56(6):399-406.

285. Suarez K, Mayer C, Ehlert U, Nater UM. Psychological stress and self-reported functional gastrointestinal disorders. J Nerv Ment Dis 2010, Mar;198(3):226-9.

286. Shaw G, Srivastava ED, Sadlier M, Swann P, James JY, Rhodes J. Stress management for irritable bowel syndrome: A controlled trial. Digestion 1991;50(1):36-42.

287. Jarrett ME, Cain KC, Burr RL, Hertig VL, Rosen SN, Heitkemper MM. Comprehensive self-management for irritable bowel syndrome: Randomized trial of in-person vs. Combined in-person and telephone sessions. Am J Gastroenterol 2009, Dec;104(12):3004-14.

288. Guthrie E, Creed F, Dawson D, Tomenson B. A controlled trial of psychological treatment for the irritable bowel syndrome. 1991, Feb;100(2):450-7.

289. Chang L, Sundaresh S, Elliott J, Anton PA, Baldi P, Licudine A, et al. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis in irritable bowel syndrome. Neurogastroenterol Motil 2008.

290. Dinan TG, Quigley EM, Ahmed SM, Scully P, O'Brien S, O'Mahony L, et al. Hypothalamic-Pituitary-Gut axis dysregulation in irritable bowel syndrome: Plasma cytokines as a potential biomarker? Gastroenterology 2006, Feb;130(2):304-11.

291. Posserud I, Agerforz P, Ekman R, Björnsson ES, Abrahamsson H, Simrén M. Altered visceral perceptual and neuroendocrine response in patients with irritable bowel syndrome during mental stress. Gut 2004, Aug;53(8):1102-8.

292. Tillisch K, Mayer EA, Labus JS, Stains J, Chang L, Naliboff BD. Sex specific alterations in autonomic function among patients with irritable bowel syndrome. Gut 2005, Oct;54(10):1396-401.

293. Chang L. The role of stress on physiologic responses and clinical symptoms in irritable bowel syndrome. Gastroenterology 2011, Jan 19;In Press, Uncorrected Proof:-.

294. Videlock EJ, Adeyemo M, Licudine A, Hirano M, Ohning G, Mayer M, et al. Childhood trauma is associated with hypothalamic-pituitary-adrenal axis responsiveness in irritable bowel syndrome. Gastroenterology 2009;137(6):1954 - 1962.

295. Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu Rev Neurosci 2001;24:1161-92.

296. Gareau MG, Jury J, Yang PC, MacQueen G, Perdue MH. Neonatal maternal separation causes colonic dysfunction in rat pups including impaired host resistance. Pediatr Res 2006, Jan;59(1):83-8.

297. Gareau MG, Jury J, Perdue MH. Neonatal maternal separation of rat pups results in abnormal cholinergic regulation of epithelial permeability. Am J Physiol Gastrointest Liver Physiol 2007, Jul;293(1):G198-203.

298. Francis D, Diorio J, Liu D, Meaney MJ. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. Science 1999, Nov 5;286(5442):1155-8.

299. Bird A. Perceptions of epigenetics. Nature 2007, May 24;447(7143):396-8.

300. Champagne F, Meaney MJ. Like mother, like daughter: Evidence for non-genomic transmission of parental behavior and stress responsivity. Prog Brain Res 2001;133:287-302.

301. Szyf M, Weaver I, Meaney M. Maternal care, the epigenome and phenotypic differences in behavior. Reprod Toxicol 2007, Jul;24(1):9-19.

302. Szyf M, McGowan P, Meaney MJ. The social environment and the epigenome. Environ Mol Mutagen 2008, Jan;49(1):46-60.

303. Grillon C. Startle reactivity and anxiety disorders: Aversive conditioning, context, and neurobiology. Biol Psychiatry 2002, Nov 15;52(10):958-75.

304. Naliboff BD, Waters AM, Labus JS, Kilpatrick L, Craske MG, Chang L, et al. Increased acoustic startle responses in IBS patients during abdominal and nonabdominal threat. Psychosom Med 2008, Oct 8;70(8):920-7.

305. LeDoux J. The emotional brain, fear, and the amygdala. Cell Mol Neurobiol 2003, Oct;23(4-5):727-38.

306. Davis M. The role of the amygdala in fear and anxiety. Annu Rev Neurosci 1992;15:353-75.

307. Davis M. Neurobiology of fear responses: The role of the amygdala. J Neuropsychiatry Clin Neurosci 1997;9(3):382-402.

308. Neugebauer V, Li W, Bird GC, Han JS. The amygdala and persistent pain. Neuroscientist 2004, Jun;10(3):221-34.

309. Bradley MM, Cuthbert BN, Lang PJ. Affect and the startle reflex. Startle Modification: Implications for Neuroscience, Cognitive Science, and Clinical Science 1999:157-83.

310. Rhudy JL, Williams AE, McCabe KM, Nguyen MA, Rambo P. Affective modulation of nociception at spinal and supraspinal levels. Psychophysiology 2005, Sep;42(5):579-87.

311. Choi JS, Kim JJ. Amygdala regulates risk of predation in rats foraging in a dynamic fear environment. Proc Natl Acad Sci U S A 2010, Nov 29.

312. De Martino B, Camerer CF, Adolphs R. Amygdala damage eliminates monetary loss aversion. Proc Natl Acad Sci U S A 2010, Feb 23;107(8):3788-92.

313. Liberzon I, Britton JC, Phan KL. Neural correlates of traumatic recall in posttraumatic stress disorder. Stress 2003, Sep;6(3):151-6.

314. Shin LM, Orr SP, Carson MA, Rauch SL, Macklin ML, Lasko NB, et al. Regional cerebral blood flow in the amygdala and medial prefrontal cortex during traumatic imagery in male and female vietnam veterans with PTSD. Arch Gen Psychiatry 2004, Feb;61(2):168-76.

315. Naliboff BD, Derbyshire SW, Munakata J, Berman S, Mandelkern M, Chang L, Mayer EA. Cerebral activation in patients with irritable bowel syndrome and control subjects during rectosigmoid stimulation. Psychosom Med 2001;63(3):365-75.

316. Naliboff BD, Berman S, Suyenobu B, Labus JS, Chang L, Stains J, et al. Longitudinal change in perceptual and brain activation response to visceral stimuli in irritable bowel syndrome patients. Gastroenterology 2006, Aug;131(2):352-65.

317. Petrovic P, Dietrich T, Fransson P, Andersson J, Carlsson K, Ingvar M. Placebo in emotional processing--induced expectations of anxiety relief activate a generalized modulatory network. Neuron 2005, Jun 16;46(6):957-69.

318. Berman SM, Naliboff BD, Suyenobu B, Labus JS, Stains J, Ohning G, et al. Reduced brainstem inhibition during anticipated pelvic visceral pain correlates with enhanced brain response to the visceral stimulus in women with irritable bowel syndrome. J Neurosci 2008, Jan 9;28(2):349-59.

319. Mayer EA, Naliboff BD, Craig AD. Neuroimaging of the brain-gut axis: From basic understanding to treatment of functional GI disorders. Gastroenterology 2006, Dec;131(6):1925-42.

320. Gue M, Junien JL, Bueno L. Conditioned emotional response in rats enhances colonic motility through the central release of corticotropin-releasing factor. Gastroenterology 1991, Apr;100(4):964-70.

321. Young SJ, Alpers DH, Norland CC, Woodruff RA. Psychiatric illness and the irritable bowel syndrome. Practical implications for the primary physician. Gastroenterology 1976, Feb;70(2):162-6.

322. Miller AR, North CS, Clouse RE, Wetzel RD, Spitznagel EL, Alpers DH. The association of irritable bowel syndrome and somatization disorder. Ann Clin Psychiatry 2001, Mar;13(1):25-30.

323. North CS, Downs D, Clouse RE, Alrakawi A, Dokucu ME, Cox J, et al. The presentation of irritable bowel syndrome in the context of somatization disorder. Clin Gastroenterol Hepatol 2004, Sep;2(9):787-95.

324. Lydiard RB, Fossey MD, Marsh W, Ballenger JC. Prevalence of psychiatric disorders in patients with irritable bowel syndrome. Psychosomatics 1993;34(3):229-34.

325. American Academy of Family Physicians APAAPAWGOD-I, American Academy of Family Physicians APAAPAWGOD-I, American Academy of Family Physicians APAAPAWGOD-I. Diagnostic and statistical manual of mental disorders, fourth edition : Primary care version. Washington, DC: American Psychiatric Association; 1995.

326. Heinrich TW. Medically unexplained symptoms and the concept of somatization. WMJ 2004;103(6):83-7.

327. Ketterer MW, Buckholtz CD. Somatization disorder. J Am Osteopath Assoc 1989, Apr;89(4):489-90, 495-9.

328. Lenze EJ, Miller AR, Munir ZB, Pornnoppadol C, North CS. Psychiatric symptoms endorsed by somatization disorder patients in a psychiatric clinic. Ann Clin Psychiatry 1999, Jun;11(2):73-9.

329. Munakata J, Naliboff B, Harraf F, Kodner A, Lembo T, Chang L, et al. Repetitive sigmoid stimulation induces rectal hyperalgesia in patients with irritable bowel syndrome. Gastroenterology 1997, Jan;112(1):55-63.

330. Tougas G, Hudoba P, Fitzpatrick D, Hunt RH, Upton AR. Cerebral-Evoked potential responses following direct vagal and esophageal electrical stimulation in humans. Am J Physiol 1993, Mar;264(3 Pt 1):G486-91.

331. Bromm B, Treede RD. Human cerebral potentials evoked by CO2 laser stimuli causing pain. Exp Brain Res 1987;67(1):153-62.

332. Bromm B, Treede RD. Pain related cerebral potentials: Late and ultralate components. Int J Neurosci 1987, Mar;33(1-2):15-23.

333. Bromm B, Lorenz J. Neurophysiological evaluation of pain. Electroencephalogr Clin Neurophysiol 1998, Oct;107(4):227-53.

334. Hobson AR, Furlong PL, Worthen SF, Hillebrand A, Barnes GR, Singh KD, Aziz Q. Real-Time imaging of human cortical activity evoked by painful esophageal stimulation. Gastroenterology 2005, Mar;128(3):610-9.

335. Prior A, Serial E, Sun WM, Read NW. Irritable bowel syndrome: Differences between patients who show rectal sensitivity and those who do not. Eur J Gastroenterol Hepatol 1993;5(5):343.

336. Mertz H, Naliboff B, Munakata J, Niazi N, Mayer EA. Altered rectal perception is a biological marker of patients with irritable bowel syndrome. Gastroenterology 1995, Jul;109(1):40-52.

337. Prior A, Maxton DG, Whorwell PJ. Anorectal manometry in irritable bowel syndrome: Differences between diarrhoea and constipation predominant subjects. Gut 1990, Apr;31(4):458-62.

338. Ritchie J. Pain from distension of the pelvic colon by inflating a balloon in the irritable colon syndrome. Gut 1973, Feb;14(2):125-32.

339. Kellow JE, Delvaux M, Azpiroz F, Camilleri M, Quigley EM, Thompson DG. Principles of applied neurogastroenterology: Physiology/motility-sensation. Gut 1999, Sep;45 Suppl 2:II17-24.

340. Bouin M, Plourde V, Boivin M, Riberdy M, Lupien F, Lagani\vcre M, et al. Rectal distention testing in patients with irritable bowel syndrome: Sensitivity, specificity, and predictive values of pain sensory thresholds. Gastroenterology 2002;122(7):1771-7.

341. Whitehead WE, Palsson OS. Is rectal pain sensitivity a biological marker for irritable bowel syndrome: Psychological influences on pain perception. Gastroenterology 1998, Nov;115(5):1263-71.

342. Gershon MD. Nerves, reflexes, and the enteric nervous system: Pathogenesis of the irritable bowel syndrome. J Clin Gastroenterol 2005;39(5 Suppl 3):S184-93.

343. Cervero F. Sensory innervation of the viscera: Peripheral basis of visceral pain. Physiol Rev 1994, Jan;74(1):95-138.

344. Cervero F, Laird JM. Understanding the signaling and transmission of visceral nociceptive events. J Neurobiol 2004, Oct;61(1):45-54.

345. Longhurst JC, Dittman LE. Hypoxia, bradykinin, and prostaglandins stimulate ischemically sensitive visceral afferents. Am J Physiol 1987, Sep;253(3 Pt 2):H556-67.

346. Häbler HJ, Jänig W, Koltzenburg M. Activation of unmyelinated afferent fibres by mechanical stimuli and inflammation of the urinary bladder in the cat. J Physiol 1990, Jun;425:545-62.

347. Belmonte C, Cervero F. Neurobiology of nociceptors. illustrated ed. Oxford; New York: Oxford University Press; 1996.

348. Adelson DW, Wei JY, Kruger L. Warm-Sensitive afferent splanchnic c-fiber units in vitro. J Neurophysiol 1997, Jun;77(6):2989-3002.

349. Bouin M, Delvaux M, Blanc C, Lagier E, Delisle MB, Fioramonti J, et al. Intrarectal injection of glycerol induces hypersensitivity to rectal distension in healthy subjects without modifying rectal compliance. Eur J Gastroenterol Hepatol 2001, May;13(5):573-80.

350. Verne GN, Robinson ME, Vase L, Price DD. Reversal of visceral and cutaneous hyperalgesia by local rectal anesthesia in irritable bowel syndrome (IBS) patients. Pain 2003, Sep;105(1-2):223-30.

351. Accarino AM, Azpiroz F, Malagelada JR. Selective dysfunction of mechanosensitive intestinal afferents in irritable bowel syndrome. Gastroenterology 1995, Mar;108(3):636-43.

352. Fukudo S, Kanazawa M, Kano M, Sagami Y, Endo Y, Utsumi A, et al. Exaggerated motility of the descending colon with repetitive distention of the sigmoid colon in patients with irritable bowel syndrome. J Gastroenterol 2002, Nov;37 Suppl 14:145-50.

353. Bielefeldt K, Christianson JA, Davis BM. Basic and clinical aspects of visceral sensation: Transmission in the CNS. Neurogastroenterol Motil 2005, Aug;17(4):488-99.

354. Giamberardino MA. Recent and forgotten aspects of visceral pain. Eur J Pain 1999, Jun;3(2):77-92.

355. Ruch TC. Howell's textbook of physiology. Fulton J, editors. ; 1946.

356. Sato A, Schmidt RF. Somatosympathetic reflexes: Afferent fibers, central pathways, discharge characteristics. Physiol Rev 1973, Oct;53(4):916-47.

357. Craig AD. How do you feel? Interoception: The sense of the physiological condition of the body. Nat Rev Neurosci 2002, Aug;3(8):655-66.

358. Craig AD. Pain mechanisms: Labeled lines versus convergence in central processing. Annu Rev Neurosci 2003;26:1-30.

359. Bernstein CN, Frankenstein UN, Rawsthorne P, Pitz M, Summers R, McIntyre MC. Cortical mapping of visceral pain in patients with GI disorders using functional magnetic resonance imaging. Am J Gastroenterol 2002, Feb;97(2):319-27.

360. Lawal A, Kern M, Sidhu H, Hofmann C, Shaker R. Novel evidence for hypersensitivity of visceral sensory neural circuitry in irritable bowel syndrome patients. Gastroenterology 2006, Jan;130(1):26-33.

361. Azpiroz F, Bouin M, Camilleri M, Mayer EA, Poitras P, Serra J, Spiller RC. Mechanisms of hypersensitivity in IBS and functional disorders. Neurogastroenterology and Motility 2007;19(s1):62-88.

362. Mayer EA, Berman S, Suyenobu B, Labus J, Mandelkern MA, Naliboff BD, Chang L. Differences in brain responses to visceral pain between patients with irritable bowel syndrome and ulcerative colitis. Pain 2005, Jun;115(3):398-409.

363. Orrison WW. Neuroimaging. illustrated ed. Philadelphia: W.B. Saunders; 2000.

364. Dorn SD, Palsson OS, Thiwan SI, Kanazawa M, Clark WC, van Tilburg MA, et al. Increased colonic pain sensitivity in irritable bowel syndrome is the result of an increased tendency to report pain rather than increased neurosensory sensitivity. Gut 2007, Sep;56(9):1202-9.

365. Hobson AR, Sarkar S, Furlong PL, Thompson DG, Aziz Q. A cortical evoked potential study of afferents mediating human esophageal sensation. Am J Physiol Gastrointest Liver Physiol 2000, Jul;279(1):G139-47.

366. Spiller RC, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner M, Neal KR. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute campylobacter enteritis and in post-dysenteric irritable bowel syndrome. Gut 2000, Dec;47(6):804-11.

367. Liebregts T, Adam B, Bredack C, Röth A, Heinzel S, Lester S, et al. Immune activation in patients with irritable bowel syndrome. Gastroenterology 2007, Mar;132(3):913-20.

368. Guglielmo-Viret V, Thullier P. Comparison of an electrochemiluminescence assay in plate format over a colorimetric ELISA, for the detection of ricin B chain (RCA-B). J Immunol Methods 2007, Dec 1;328(1-2):70-8.

369. Blalock JE, Smith EM. Conceptual development of the immune system as a sixth sense. Brain Behav Immun 2007, Jan;21(1):23-33.

370. Ader R, Cohen N. Behaviorally conditioned immunosuppression. Psychosom Med 1975;37(4):333-40.

371. Segerstrom SC, Miller GE. Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. Psychological Bulletin 2004;130(4):601-30.

372. Avitsur R, Padgett DA, Sheridan JF. Social interactions, stress, and immunity. Neurol Clin 2006, Aug;24(3):483-91.

373. Elliot, G. R, Eisdorfer, C.. Stress and human health: Analysis and implications of research : A study. Springer Pub. Co.; 1982.

374. Kiecolt-Glaser JK, Dura JR, Speicher CE, Trask OJ, Glaser R. Spousal caregivers of dementia victims: Longitudinal changes in immunity and health. Psychosom Med 1991;53(4):345-62.

375. Herbert TB, Cohen S. Stress and immunity in humans: A meta-analytic review. Psychosom Med 1993;55(4):364-79.

376. Cohen S, Miller GE, Rabin BS. Psychological stress and antibody response to immunization: A critical review of the human literature. Psychosom Med 2001;63(1):7-18.

377. Dopp JM, Miller GE, Myers HF, Fahey JL. Increased natural killer-cell mobilization and cytotoxicity during marital conflict. Brain Behav Immun 2000, Mar;14(1):10-26.

378. Sapolsky RM. Why zebras don't get ulcers. New York: Times Books; 2004.

379. Marshall GD, Agarwal SK, Lloyd C, Cohen L, Henninger EM, Morris GJ. Cytokine dysregulation associated with exam stress in healthy medical students. Brain Behav Immun 1998, Dec;12(4):297-307.

380. Jones JF. An extended concept of altered self: Chronic fatigue and post-infection syndromes. Psychoneuroendocrinology 2008, Feb;33(2):119-29.

381. Hart BL. Biological basis of the behavior of sick animals. Neurosci Biobehav Rev 1988;12(2):123-37.

382. Peters A, Schweiger U, Pellerin L, Hubold C, Oltmanns KM, Conrad M, et al. The selfish brain: Competition for energy resources. Neurosci Biobehav Rev 2004, Apr;28(2):143-80.

383. Taché Y, Brunnhuber S. From hans selye's discovery of biological stress to the identification of corticotropin-releasing factor signaling pathways: Implication in stress-related functional bowel diseases. Ann N Y Acad Sci 2008, Dec;1148:29-41.

384. Taché Y, Kiank C, Stengel A. A role for corticotropin-releasing factor in functional gastrointestinal disorders. Curr Gastroenterol Rep 2009, Aug;11(4):270-7.

385. Taché Y, Martinez V, Million M, Maillot C. Role of corticotropin releasing factor receptor subtype 1 in stress-related functional colonic alterations: Implications in irritable bowel syndrome. Eur J Surg Suppl 2002(587):16-22.

386. Fukudo S, Nomura T, Hongo M. Impact of corticotropin-releasing hormone on gastrointestinal motility and adrenocorticotropic hormone in normal controls and patients with irritable bowel syndrome. Gut 1998, Jun;42(6):845-9.

387. Sagami Y, Shimada Y, Tayama J, Nomura T, Satake M, Endo Y, et al. Effect of a corticotropin releasing hormone receptor antagonist on colonic sensory and motor function in patients with irritable bowel syndrome. Gut 2004, Jul;53(7):958-64.

388. Ströhle A, Holsboer F. Stress responsive neurohormones in depression and anxiety. Pharmacopsychiatry 2003, Nov;36 Suppl 3:S207-14.

389. Bremner JD, Licinio J, Darnell A, Krystal JH, Owens MJ, Southwick SM, et al. Elevated CSF corticotropin-releasing factor concentrations in posttraumatic stress disorder. American Journal of Psychiatry 1997;154(5):624.

390. Southwick SM, Vythilingam M, Charney DS. The psychobiology of depression and resilience to stress: Implications for prevention and treatment. Annu Rev Clin Psychol 2005;1:255-91.

391. Infante-Duarte C, Horton HF, Byrne MC, Kamradt T. Microbial lipopeptides induce the production of IL-17 in th cells. J Immunol 2000, Dec 1;165(11):6107-15.

392. Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, Fouser LA. Interleukin (IL)-22 and IL-17 are coexpressed by th17 cells and cooperatively enhance expression of antimicrobial peptides. J Exp Med 2006, Oct 2;203(10):2271-9.

393. Steinman L, Steinman L. A brief history of TH17, the first major revision in the TH1/TH2 hypothesis of T cell|[ndash]|mediated tissue damage. Nat Med 2007, Feb 2;13(2):139.

394. Domingues HS, Mues M, Lassmann H, Wekerle H, Krishnamoorthy G. Functional and pathogenic differences of th1 and th17 cells in experimental autoimmune encephalomyelitis. Plos One 2010;5(11):e15531.

395. Wynn TA. T(H)-17: A giant step from T(H)1 and T(H)2. Nat Immunol 2005, Nov;6(11):1069-70.

396. Kolls JK, Lindén A. Interleukin-17 family members and inflammation. Immunity 2004, Oct;21(4):467-76.

397. Reinhardt RL, Kang SJ, Liang HE, Locksley RM. T helper cell effector fates--who, how and where? Curr Opin Immunol 2006, Jun;18(3):271-7.

398. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol 2005, Nov;6(11):1133-41.

399. Andoh A, Ogawa A, Bamba S, Fujiyama Y. Interaction between interleukin-17-producing CD4+ T cells and colonic subepithelial myofibroblasts: What are they doing in mucosal inflammation? J Gastroenterol 2007, Jan;42 Suppl 17:29-33.