From nociception to perception

SUMMARY

Chronic visceral pain is a hallmark symptom of many disorders of gut-brain interaction (DGBI). These disorders can be characterized by upper abdominal pain, such as in functional dyspepsia (FD), or lower abdominal pain, such as in irritable bowel syndrome (IBS). Although both FD and IBS are highly common with reported prevalence rates in Western populations of around 10%, their treatment remains challenging. The complexity of mechanisms involved in generating the subjective sensation of visceral pain from a stimulus highlights the difficulty of the development of effective treatment strategies.

In this thesis, we approach visceral pain mechanisms and abnormalities leading to increased pain sensitivity from both a peripheral and central perspective. To this end, the current thesis is divided into three major parts. The first part focusses on molecular transducers of visceral pain, in particular transient receptor potential channels. The second part explores the association between hypermobility spectrum disorders (i.e. hereditary non-inflammatory connective tissue abnormalities characterized by increased mobility of joints) and visceral pain. The third part describes central pain processing, pain perception and measurement of visceral pain symptoms.

Part I - molecular transducers of visceral pain

In **chapter 2**, we review current literature regarding the role of the different transient receptor potential (TRP) channels, i.e. TRPV1, TRPV4, TRPA1 and TRPM8 in IBS. Per TRP channel, we provide an overview of gastrointestinal expression patterns, mechanisms of activation and sensitization, and their putative implication in chronic visceral pain in IBS. Possible therapeutic targets were highlighted. We finally described various mechanisms of TRP channel targeted therapy, including (selective) antagonism, (cross-)desensitization, downstream targeted therapy and, RNA-based therapy (experimental). Given the important role of TRP channels in visceral pain generation and inhibition, and the various options for regulating their function, it seems likely that TRP channel targeted therapy will become an effective option for visceral pain management in IBS and other DGBIs. The primary challenge in treatment development is related to the large amount of physiological functions of TRP channels. Expanding our knowledge on TRP channel functioning in (patho)physiological conditions will aid future treatment development.

In order to find potential analogies and/or differences in states of chronic intestinal inflammation, we reviewed current literature regarding TRPV1 and TRPA1 in inflammatory bowel disease (IBD) in **chapter 3**. TRPV1 and TRPA1 not only play a complex role in hyperalgesia, but also in (neurogenic) inflammation. Regarding the latter, the role of these ion channels in IBD is seemingly contradictory. Activation of

these receptors on sensory nerve terminals mediates neurogenic inflammation via the release of sensory neuropeptides (such as substance P and calcitonin gene-related peptide, CGRP), resulting in increased vascular permeability and inflammatory cell activation. Meanwhile, anti-inflammatory sensory neuropeptides, such as somatostatin and opioid peptides released simultaneously from the same nerve ending exert anti-inflammatory and analgesic actions both locally and systemically. It remains to be elucidated which processes result in tipping the scale to a net pro-inflammatory or anti-inflammatory effect. Till date however, preclinical studies more convincingly point out the potential therapeutic value of TRPV1 and TRPA1 antagonists in colitis and visceral hypersensitivity.

In **chapter 4**, we explored age-related changes in visceral nociception and related alterations in TRP channels, as this natural analgesic effect could serve as an example in the development of new treatment strategies. Previous studies pointed to a decrease in abdominal pain symptoms with ageing. Our study corroborated these findings, showing lower abdominal pain scores in elderly versus young adults in both healthy volunteers and IBS patients. We furthermore found that visceral hypersensitivity, as assessed using a rectal barostat procedure, was significantly less common in elderly IBS patients as compared to young adults. Investigating possible underlying mechanisms, we found that relative TRPA1 gene transcription, as well as TRPA1 and TRPV1 immunoreactivity were significantly lower in healthy elderly versus healthy young adults. We therefore concluded that decreased visceral sensitivity in elderly may be attributed to decreased TRPA1 and/or TRPV1 receptor expression in the intestinal epithelium. Future studies will need to assess these biological changes on a larger scale and include assessments for neural density, as neurodegeneration could also have role in the decreased visceral sensitivity in elderly.

Part II - visceral pain in hypermobility spectrum disorders

Abdominal symptoms and DGBIs are highly prevalent among individuals with hypermobility spectrum disorders (HSD). In **chapter 5**, we provide a review of current literature on abdominal symptoms in joint hypermobility syndrome/Ehlers-Danlos hypermobility type (now commonly referred to as HSD). Although a wide range of abdominal symptoms was reported in HSD patients, upper gastrointestinal symptoms, including bloating and postprandial fullness appeared to be the most common. Underlying mechanisms remain to be elucidated. Unfortunately, few studies have been performed to increase our understanding of GI physiology alterations in HSD. Two recent studies in FD patients by Carbone et al. did not identify gastric motility or sensitivity differences when dividing groups in HSD and non-HSD patients. We can therefore only speculate regarding the underlying mechanisms of abdominal symptoms in HSD. We hypothesized that changes in afferent signaling (e.g. due to extracellular matrix alterations) result in increased peripheral discharge of nociceptive information

(peripheral sensitization) and/or augmentation of afferent information at the level of spinal dorsal horn neurons (central sensitization). More in-depth mechanistic studies are required to investigate these hypotheses.

Anecdotal evidence suggests that colonoscopy is more difficult in individuals with HSD, possibly due to laxity of intestinal tissue and increased pain sensitivity. In **chapter 6**, we describe a prospective exploratory study performed at the endoscopy unit, reporting on pain scores during colonoscopy and cecal intubation time in HSD and non-HSD individuals. A total of 200 patients was included, of whom 22 (11%) met criteria for HSD. Although initial linear regression demonstrated higher pain scores in HSD patients, this was found to be related to a confounding effect of female gender. After correction for confounding factors, we found no differences in pain scores, cecal intubation time or endoscopist-reported procedural difficulty. It should be noted that the number of HSD patients was relatively low, however. Moreover, the sedative of choice at the endoscopy unit, midazolam, is known to cause anterograde amnesia in a subset of patients, hence patient may not fully recall their experience after colonoscopy. For assessing visceral pain sensitivity in HSD, different approaches will be necessary. Nonetheless we conclude that HSD status appears irrelevant in colonoscopy practice.

In **chapter 7**, we assess characteristics of IBS patients (n = 258) with and without HSD, with a focus on GI symptomatology, affective symptoms and perceived quality of life. When comparing IBS patients with HSD to their non-HSD counterparts, no apparent differences in gastrointestinal symptoms, depression, anxiety, and quality of life were found. Future studies should focus on differences in additional somatic symptoms (including overlapping DGBIs), autonomic symptoms, and somatization scores, in order to unravel the question of whether IBS patients with a comorbid diagnosis of joint hypermobility or HSD represent a specific subgroup within the IBS population.

Part III – visceral pain processing and perception

In the third part of this thesis, we report on several studies at the level of central pain processing and perception, i.e. measurement of symptoms in clinical trials. **Chapter 8** describes a neuroimaging study in 18 healthy volunteers using a novel visceral pain model consisting of the duodenal infusion of capsaicin. During infusion, functional magnetic resonance imaging (fMRI) at ultrahigh field strength (7 Tesla) was used to investigate brain responses, with a particular focus on the nucleus of the solitary tract (NTS), the primary relay station of visceral afferents in the brainstem. Significantly increased brain activation over time during capsaicin infusion, as compared to placebo, was observed in brain regions implicated in pain processing, in particular the NTS. Brain activation in the thalamus, cingulate cortex and insula were more pronounced in subjects who reported abdominal pain (visual analogue scale > 10mm), as compared to subjects who experienced no pain. On the contrary, activations at the level of the NTS

were independent of subjective pain ratings. These findings prompt the exploration of fundamental mechanisms related to how pain emerges from nociception as well as new therapeutic approaches to treating visceral pain conditions, for instance, by targeting specific areas within the gut–brain axis to downregulate disturbed visceroception and hyperalgesia.

From a methodological point of view, fMRI experiments are inherently difficult. Most notably, it can be challenging to develop a valid data preprocessing pipeline, which is necessary to enable robust analyses. In **chapter 9**, we describe the technical aspects of quality of several data cleaning approaches in order to increase temporal signal-to-noise ratio (tSNR; among other data quality metrics). Optimized tSNR is of particular interest in brainstem neuroimaging, as large vessel and air-filled cavities proximity results in a generally lower tSNR as compared to cortical brain regions. Using our imaging paradigm described in **chapter 8**, which included a multi-echo sequence optimized for brainstem imaging, we compared several data cleaning approaches. Optimal results were achieved with multi-echo independent component analysis (ME-ICA) combined with anatomical component based correction (aCompCor), which was also our approach of choice in **chapter 8**.

The final step after central pain processing entails the experience of pain. Given its subjective nature, high quality measurement instruments are necessary to provide the best representation of patient symptoms. **Chapter 10** describes a comparative study of digital instruments to measure gastrointestinal symptoms. In this study, we included 25 IBS patients and 15 FD patients. All subjects were required to have participated in a randomized controlled trial (which incorporated a digital end-of-day diary) and observational study (which used momentary assessments, i.e. the experience sampling method [ESM]). We found excellent adherence rates for the end-of-day diaries as well as good adherence to the ESM-based applications. Overall adherence rates for ESM were evidently lower, as would be expected given the nature of the methodology (and consequently, higher patient burden). Nonetheless, ESM does have added value over end-of-day diaries due to the frequent and more extensive measurements. Researchers should choose between these sampling methods based on their research questions, or try to combine them optimally (e.g. use ESM at intervals).

Finally, we summarize the main findings of this thesis in **chapter 11**, highlighting future implications and possibilities for further research.