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Studying the Brain Gut Axis with Pharmacological Imaging

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Abstract

Pharmacological imaging provides great potential both for evaluating the efficacy of new candidate compounds in the treatment of gastrointestinal symptom-based disorders, and for furthering our understanding of the underlying pathophysiology of such disorders. By combining evaluation of symptoms, behavior, and brain responses to relevant stimuli, use of neuroimaging is able to move the study of brain-gut disorders away from more subjective outcomes and emphasize the underlying neural networks involved in symptom generation and treatment. This chapter reviews the state of the art in pharmacological imaging studies, both in human subjects and in animal models of brain gut interactions.

Keywords

Irritable Bowel Syndrome; Brain-Gut axis; neuroimaging; functional gastrointestinal disorder

Introduction

The application of different neuroimaging techniques to drug discovery and candidate compound evaluation has great potential for many symptom based disorders, including functional GI disorders.[1] Such techniques include molecular imaging with Positron Emission Tomography (PET) and PET ligands, functional magnetic resonace imaging ("pharmacological fMRI") , and conventional techniques to study neural activity in animal models.[2-5] TABLE 1 In addition to providing information about structure, function, and molecular signaling within the brain, this combined approach may improve the translation from animal models of these disorders to the human syndrome[6], potentially expediting the decision making process for candidate compounds likely to be effective in human patients. By evaluating a compound's effect on brain function in both animal models and human patients, this translational imaging approach avoids the difficult challenge of linking a particular animal behavior to a human subjective symptom.

The assessment of symptoms and their improvement with therapeutic interventions in patients with functional GI disorders depends currently on the subjective patient reports

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obtained in lengthy and expensive phase II and III clinical trials. The need for large patient numbers in these studies, requiring many participating centers, often in different parts of the world, further increases the variability of subjective data. The use of functional imaging techniques, in particular fMRI, has the potential to provide sensitive and specific biomarkers (or endophenotypes) relevant to the symptom complex of functional GI syndromes, such as primary visceral afferent hypersensitivity, increased activity in central arousal systems, compromised endogenous pain inhibition systems and other pain amplification mechanisms. Functional brain imaging can provide information about both the activity of the functional connectivity within central autonomic networks, and about the effect that candidate compounds may have on these parameters.[7]

The ability to sample a specific neurobiological substrate (e.g., a specific brain region or nucleus) hundreds of times in the same subject, greatly reduces the intra- and inter-individual variability of the measure. Thus, neuroimaging-based evaluation of drug effects in small samples of patients has the potential to identify significant drug effects on a specific biomarker of the disorder, while much larger patient numbers are required to detect significant effects on subjective symptoms. Since fMRI can be applied to animal models as well, identification of drug effects in preclinical studies may have predictive validity for finding such effects in human studies as well. Several studies outside of the field of Gastroenterology have reported the ability of functional brain imaging to detect changes in brain activation induced by various pharmacological (as well as non-pharmacological) therapeutic interventions, ranging from antidepressants,[8] anxiolytics,[9, 10] opioids,[11] and gabapentin[12]to hypnosis,[13] placebo,[14-16] and cognitive behavioral therapy.[17] These results have demonstrated that several of these interventions affect activity within the homeostatic afferent processing network,[18] as well as specific circuits in the brain that play a role in arousal and in endogenous pain modulation. A particularly interesting example supporting the concept of dACC as a region involved in affect and motivational responses to painful stimuli is the biofeedback training of subjects to increase or decrease the activation of the dorsal anterior cingulate cortex (dACC), using real time fMRI.[19] Healthy subjects were successfully trained to increase or decrease pain perception to an experimental stimulus based on their volitional control of dACC activity and chronic pain patients were able to decrease their spontaneous pain by decreasing dACC activity.

Human pharmacological imaging in patients with functional GI disorders

Despite the lack of consensus regarding brain responses to rectal stimuli in healthy controls and group differences between irritable bowel syndrome (IBS) patients and control subjects, several studies have been reported using functional brain imaging to identify changes in cerebral activation associated with various treatment modalities, including pharmacological[20, 21] and non-pharmacological treatments.[16, 22] In contrast to the emerging publications in the somatic pain and psychiatric literature,[7, 10, 12, 23] only a few of the reported studies are of sufficient quality (statistical power, blinding, homogeneous study populations) to allow any conclusions from the results.

The effect of antidepressant treatment on brain activity in IBS patients

Morgan et al reported an effect of low-dose amitriptyline treatment (compared with placebo) on rectal distention–induced brain activation combined with a psychological stressor using fMRI. [24] Although the drug had no effect on symptoms or distentions alone, decreased activation in the rostral ACC (rACC) and the left posterior parietal cortex was observed during distention when associated with the psychological stressor.

5 Hydroxytryptamine (5-HT) receptor modulating drug effects on brain activity and connectivity

Berman et al reported a double-blind, randomized, placebo-controlled study in 49 IBS patients (26 women; nonconstipated) who underwent $H_2\ ^{15}O$-PET scanning before and after a 3-week course of the $5\text{-}HT_3$ receptor antagonist, alosetron. [21] Active treatment was associated with reduced blood flow in the amygdala and the ventral striatum, ventromedial PFC, and a pontine region, but not with significant changes in the homeostatic afferent network (insula, dACC, thalamus). Significant reductions were only seen at baseline and during the expectation condition, but not during the rectal distention, suggesting that the main effect of the drug was on brain networks engaged during expectation, rather than input from the gastrointestinal tract (visceral afferent pathways) during rectal distension. Supporting this interpretation was the finding that IBS symptom improvement was correlated with activity in regions of the brain associated with response to arousal (amygdala, ventral striatum, and dorsal pons) rather that from those associated with sensory input.

In a preliminary report, Kilpatrick et al used fMRI to characterize the effect of another 5-HT modulating drug, the partial 5-HT4 receptor agonist tegaserod (vs placebo) on brain responses to rectal distention in a crossover study of 10 female IBS patients. [25] Effective connectivity analysis revealed a distention-activated network of regions associated with the medial thalamus that functioned differently during tegaserod and placebo treatment. This treatment-dependent medial thalamic network included the homeostatic afferent network (insula, dACC), as well as a cortico-limbic network (amygdala, ventral ACC, and prefrontal cortices). Thus, despite the small sample size, the study results suggest drug-induced differences in the functioning of a spinal-thalamic-cortico-limbic circuit operating both during expectation and during the experience of noxious visceral stimuli.

An additional effective connectivity analysis of the data revealed *symptom*-related networks that functioned differently during tegaserod and placebo treatment. During expectation of a rectal distention, patients demonstrated stronger cortical dampening of the limbic system (as seen by greater influence of the medial orbitofrontal cortex (mOFC) on amygdala activity). Additionally, while taking tegaserod patients showed more active negative feedback on emotional arousal as evidenced by a greater negative influence of infragenual ACC (iACC) on rACC activity relative to placebo. During actual delivery of a painful rectal distention, tegaserod (relative to placebo) altered the coupling among regions central to the processing of information from the GI tract (reduced thalamic influence on rACC activity and more negative thalamic influence on mOFC and insula activity) and emotional arousal (more negative influence of iACC on rACC activity). Overall, the findings suggest that in addition to its known prokinetic effects, the drug's beneficial effect on IBS symptoms may also involve effects on brain circuits during both expectation and experience of acute rectal discomfort.

Neurokinin-1 receptor modulating drug effects on brain activity and connectivity

A double blind, placebo-controlled pilot study in women with IBS (n=10) used chronic administration of a neurokinin-1 receptor antagonist (NK1Ra), evaluating brain responses to both emotional and visceral stimuli.[26, 27] As IBS patients were hypothesized to have altered processing of emotional information, the subjects were instructed to view negative emotional faces and label the faces with the correct emotion. It has previously been reported that in this task, IBS patients compared to healthy control subjects, have decreased

activation of the ventrolateral prefrontal cortex (vlPFC).[28] These findings suggested that IBS patients show a compromised ability to down regulate the emotional response and to recruit prefrontal inhibitory regions.[29] When comparing drug versus placebo condition, patients showed significantly greater activation of the vlPFC compared to placebo condition, suggesting a normalization of prefrontal activation by the drug. A second task tested the drug's effect on response to a painful rectal balloon inflation compared to a non-painful inflation. In the drug group, subjects showed decreased pain ratings during rectal aversive distension, as measured by the McGill Total Pain Scale, and these subjective findings were associated with reduced activation of the dACC. Activity in the dACC during painful stimuli is associated with the motivational and cognitive response to pain. No differences in brain stem or insular cortical activations were seen, suggesting that the drug did not have a significant effect on afferent sensory input. The fact that significant differences in drug-placebo conditions were noted in the brain in this small sample, when many more subjects would need to be studied to show significant symptom changes, supports the use of fMRI methods for initial compound screening (phase IIa studies) prior to beginning large scale clinical trials.

Functional brain imaging in rodents and its potential for pharmacological imaging

Current preclinical evaluation of candidate compounds for visceral pain relies primarily on measuring pseudoaffective responses to colorectal distension (CRD) [30] in restrained, and sometimes sedated rodents. Typically, electromyographic (EMG) contraction of the abdominal muscles [31-33], or behavioral pain postures [34] are measured. In view of the multidimensional nature of the human pain experience, it is clear that pseudoaffective responses in rodents reflect only a small portion of the nociceptive response. To capture more objective markers of the animal visceral pain response, and to assess possible drug effects on this response, functional brain imaging has begun to be applied to compare the brain response in rodents to that in humans. Until recently, research of brain responses to visceral stimulation at the whole-brain level in rodents has relied largely on measurement of c-fos protein expression [3, 4, 35]. Results, however, have been variable between studies and without consistent parallels to human brain imaging findings. In theses studies, to elicit a robust c-fos signal, high-intensity visceral stimuli (e.g., CRD at 80 mmHg) are typically used with inflation/deflation cycles occurring repeatedly over long durations (usually more than one hour). It is likely that a variety of non-specific brain responses become integrated in the c-fos signal during this time. Furthermore, brain responses are dynamic and susceptible to such processes as habituation and sensitization that can occur with prolonged stimuli. Hence, a comparison between results from c-fos brain imaging in rodents and those from fMRI or PET imaging in humans (with a temporal resolution of milliseconds to seconds) is difficult. It is noteworthy that studies examining increases in c-fos expression in response to CRD in the lumbosacral region of the spinal cord have reported more consistent results [36-39], but parallels to human imaging have not been explored extensively. Lazovic et al. [40] conducted the only reported fMRI study in the rat CRD model. fMRI scans were acquired in anesthetized rats during acute application of CRD (45 to 80 mmHg). Significant activation were seen in limbic and paralimbic areas, including the amygdala, hypothalamus, thalamus, and hippocampus, with variable responses in the cerebral cortex (e.g. CRD evokes insular activation in 4 out of 9 animals). This variable cortical activation may be attributable to the use of anesthetized animals. Importantly, the authors compared regional brain activation measured with fMRI and that measured with c-fos expression, and showed that these techniques reveal largely different regional brain activation.

More recently, Ohashi et al. [41] reported a microPET study assessing CRD-induced brain activation in a rat model of visceral hypersensitivity. Regional brain activation was

quantified by measuring ^{18}F-fluorodeoxyglucose (FDG) uptake after CRD. CRD of a subthreshold intensity (0 to 35 mmHg) produced pain postures in rats treated with 2,4,6-trinitrobenzene sulfonic acid, as well as increases in FDG uptake in the thalamus and primary sensory cortex. Morphine attenuated activation in both brain regions in response to CRD in the sensitized rats. Since imaging of brain metabolism using FDG typically takes place after tracer uptake is complete and relatively imperturbable, this method is suitable for neuroimaging in nontethered, ambulatory subjects. The primary drawback of FDG is that the duration of the uptake and capture of the tracer is around 25-45 minutes, which requires prolonged CRD stimulation protocols. Furthermore, the spatial resolution, with micro-PET and advanced image reconstruction software, remains at best ~1.2 mm at the center of the field of view. This represents ~7% and 13% of the width of the rat and mouse brain, respectively and is poorly suited for the detection for all but the broadest changes in regional cerebral blood flow or metabolism.

Wang et al. have recently begun to examine functional brain activation in rats in response to noxious CRD with perfusion mapping, in which radiotracer (^{14}C-iodoantipyrine) is injected in the awake, nonrestrained animal during CRD and brain mapping is achieved by analyzing changes in regional cerebral blood flow (rCBF)-related tissue radioactivity using statistical parametric mapping [42]. This autoradiographic method allows one to examine regional brain activation across the entire brain, with a spatial resolution (~100 microns) appropriate for the rat or mouse brain, and a temporal resolution (seconds) sufficient for capturing acute brain changes. Results showed that many of the regions implicated in nociceptive processing of visceral stimulation in humans demonstrate significant changes in rCBF in the rat CRD model. Noxious CRD at 60 mmHg in male rats elicited significant increases in rCBF in sensory (insula, somatosensory cortex), as well as limbic and paralimbic regions (including ACC and amygdala). Significant decreases in rCBF were seen in the thalamus, parabrachial nucleus, periaqueductal gray, hypothalamus and pons. Correlations of rCBF with simultaneously measured EMG and with behavioral pain scores were noted in the anterior cingulate, insular, somatosensory and motor cortices, as well as in the lateral amygdala and dorsal caudate putamen. However, not all regions demonstrating significant group differences correlated with EMG or behavioral measures, suggesting that functional brain imaging captures more extensive nociceptive responses to noxious visceral stimulation than those identified by traditional measures. Ongoing work exploring sex-differences of this response, in parallel with studies in humans [43-45], shows that male rats have a broader cortical activation in response to noxious CRD than females, while females have a more widespread activation of subcortical structures, in particular in limbic/paralimbic regions [46]. In addition, early work suggests that brain regions implicated in visceral pain processing and modulation in human subjects are also activated in expectation of CRD in rats in a step-down passive avoidance paradigm [47]. Possibilities exist in the future for applying connectivity analysis to these data sets to understand brain responses as the network level [48].

Functional brain mapping in rodents likely will complement behavioral measurements in animal models of visceral pain. Brain mapping in rodents is beginning to validate the relevance of animal models to human conditions at the brain level. Brain mapping in transgenic and knockout mouse models holds promise for improving our understanding of the role genes play in modulating the brain's response to visceral pain. Early work suggests that imaging may be a useful tool for preclinical evaluation of candidate drugs [41, 49], the results of which may predict similar changes in humans. Future studies will have to address the predictive validity of this premise.

Summary and conclusions

The information gained from a small number of published neuroimaging studies of brain activity associated with treatment responses in IBS patients has to be considered as preliminary. The finding of selective effects of alosetron treatment on limbic, but not primary pain regions, and the correlation of these limbic effects with IBS symptom ratings demonstrates the potential strength of this technique to understanding the action of new IBS treatments. Well-designed treatment studies, with adequate sample size, homogeneous study populations, and reproducible study paradigms are needed to confirm the validity of this approach to monitor treatment effects, and predict possible clinical outcomes. However, the potential benefit of the pharmacological brain imaging approach to drug discovery and evaluation is considerable.[50] Candidate compounds aimed at ascending and descending modulatory influences on the homeostatic afferent processing network can be evaluated in their effectiveness and pharmacokinetics on animal models, and on their clinical relevance in small early phase II studies.

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TABLE 1

Comparison of brain imaging modalities used for drug development in brain-gut disorders

Imaging modality	Method	Measurement	Advantages	Disadvantages
HUMAN STUDIES				
PET	Measures emissions from radiolabeled chemical injected into the bloodstream	H_2O^{15} – blood flow F^{18} Fluorodeoxyglucose (FDG) – cellular metabolism	• Can directly show blood flow (H_2O^{15}) or glucose metabolism (FDG) in brain tissues	• Variable temporal resolution (tracer dependent) • Radiation exposure
PET ligand		Radiolabeled drug/molecule of interest	• Can observe regional receptor occupancy	• Ligand availability • Radiation exposure
fMRI	Measures magnetic variations due to the amount of oxygenated and deoxygenated hemoglobin	Blood oxygen level dependence (BOLD), which correlates with blood flow	• Noninvasive • Good spatial resolution • Good temporal resolution	• BOLD is an indirect measure of blood flow • Some regions of the brain are poorly visualized due to technical artifacts
ANIMAL STUDIES				
fMRI	Measures magnetic variations due to the amount of oxygenated and deoxygenated hemoglobin	Blood oxygen level dependence (BOLD), which correlates with blood flow	• Good spatial and temporal resolution • Able to accommodate repeated studies in a single animal	• Requires sedation and restraint of the animal to avoid movement
microPET	Measures emissions from radiolabeled tracers injected into the bloodstream	H_2O^{15} – blood flow ^{18}F Fluorodeoxyglucose (FDG) – cellular metabolism	• Uptake of radiotracer may take place in ambulatory animals (FDG) • Able to accommodate repeated studies in a single animal	• Poor spatial resolution for brain imaging in small animals • Requires sedation and restraint of the animal to avoid movement
c-fos	Evaluates early response genes	c-fos mRNA or protein	• Spatial resolution at the cellular level	• Poor temporal resolution • Poor ability for 3D mapping • Requires animal sacrifice so repeated studies cannot be performed
Autoradiography	Injection of radiotracer and exposure of the cryosectioned brain to photographic film or a phosphor imager	Cerebral blood flow, Cerebral metabolism	• Good spatial and temporal resolution • Imaging in nonrestrained, nonsedated animals	• Requires animal sacrifice so repeated studies cannot be performed.