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3D registration of micro-PET-CT for measurable correlates of dyspeptic symptoms in mice

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Abstract

Patients with chronic calorie insufficiency commonly suffer from upper gastrointestinal dysfunction and consequent dyspeptic symptoms, which may interfere with their nutritional rehabilitation. To investigate the relationship between gastric dysfunction and feeding behavior, we exposed mice to chronic caloric restriction and demonstrated gastric motor abnormalities in them. Gastric dysmotility is typically associated with dyspeptic symptoms but sensations cannot be directly assessed in animal models. Therefore, as an initial step toward establishing measurable correlates of postprandial symptoms in small animals, we have attempted to characterize central responses to food intake by positron emission tomography—computerized microtomography (PET-CT) in normal and calorically restricted mice. Animals consumed a standard test meal after an overnight fast before receiving 2-deoxy-2[¹⁸F]fluoro-D-glucose tracer. The same mice were also scanned in the fasting state on a separate day. We were able to bring the fed and fasting PET volume images into spatial registration with each other and with an MR-derived atlas of the mouse brain, so that the differences in uptake between the two states could be mapped quantitatively against the neuroanatomic regions of the atlas. Our approach is suitable for studying the effects of gastric dysmotilities on central responses to feeding.

Keywords

registration; micro-PET; micro-CT; caloric restriction; dyspepsia; anorexia nervosa; bulimia nervosa

1. INTRODUCTION

Epigastric pain, bloating, early satiety, fullness, belching, nausea, and vomiting are common in various disorders including diabetes mellitus, anorexia nervosa, a primary eating disorder

characterized by severe, self-imposed starvation, and bulimia nervosa, where uncontrolled eating binges alternate with restrictive dieting, purging or excessive exercise. The same symptoms also occur in functional dyspepsia, a clinical syndrome defined by chronic or recurrent upper abdominal symptoms without a cause identifiable by conventional diagnostic means^[1–5]. Dyspeptic symptoms are often related to, or exacerbated by, food intake and may reflect gastric motor dysfunctions or visceral hypersensitivity or both^[5, 6]. In eating disorders, postprandial dyspeptic symptoms may interfere with nutritional rehabilitation by reducing the motivation to eat and/or by perpetuating the abnormal eating behaviors and thus triggering a positive feedback cycle^[3, 4]. However, this hypothesis has never been tested formally and the underlying mechanisms remain unclear. We have established mouse models of chronic and chronic intermittent caloric restrictions and demonstrated that these mice displayed gastric motor dysfunctions characteristic of eating disorders. However, assessment of sensations in animal models requires an indirect approach, for example, functional brain imaging using positron emission tomography—computerized microtomography (PET-CT). A PET study in humans utilizing gastric balloon distension has indicated an accentuated, but regionally matched, changes in cerebral blood flow in patients with functional dyspepsia relative to healthy volunteers^[6]. However, central responses to balloon distension reflect the activation of the visceral pain neuromatrix and may differ significantly from changes induced by food intake^[7]. Therefore, as an initial step toward establishing measurable correlates of postprandial symptoms in small animals, we have attempted to characterize central responses to spontaneous food intake by PET-CT in normal and calorie-restricted mice.

2. METHODS

2.1 Animals

All studies were approved by the Institutional Animal Care and Use Committee of the Mayo Clinic. 5–7-week-old female BALB/c mice were purchased from The Jackson Laboratory and housed individually in specialized cages lined with filter paper to facilitate the recovery of uneaten food. Mice were fed ad libitum until 10–11 weeks of age. At that time, they were assigned into the following groups: Mice in group 1 were continued to be fed ad libitum (AL; n=6), whereas group 2 was offered reduced amounts of food which was continuously adjusted to keep the mice's body weights within 90–100% of their initial weights (limited-fed mice; LF; n=6). Gastric motor function was monitored by biweekly measurement of gastric emptying of solid food with the aid of a [¹³C]-octanoic acid breath test^[8]. After the development of delayed gastric emptying, 3 LF mice were placed on chronic treatment with R3 LONG recombinant human insulin-like growth factor-I (R3L-IGF-I; 150 µg/kg) injected subcutaneously at 9 AM and 5 PM, which normalized their gastric emptying within ~20 days. PET-CT scans were performed after 35–44 weeks of caloric restriction when the LF mice's body weights were ~40% lower than the weights of the AL mice and 43–45 days after the initiation of the R3L-IGF-I treatment. All mice had normal gastric emptying at the time of the PET-CT scans.

2.2 Scanning

PET-CT was performed on a Siemens INVEON scanner in the Mayo Small Animal Imaging Core. After an overnight fast, the mice ate 200 mg cooked egg yolk, then received 0.3 μCi 2-deoxy-2-[^{18}F]fluoro-D-glucose intraperitoneally to visualize metabolic activity^[9], anesthetized by isoflurane inhalation (1.5–2%) and scanned for 10 min^[10]. After 2–17 days a baseline scan was obtained the same mice after an overnight fast but without food.

2.3 Registration

Our goal in image analysis is to bring the fed and fasting PET volume images into spatial registration with each other and with an MR-derived atlas of the mouse brain^[11] as shown in figure 1, so that the differences in uptake between the two states can be mapped quantitatively against the neurologic regions of the atlas. This task is impeded by the relatively low resolution of the PET images. The atlas images, though differentiating only gross anatomic regions compared to extant human neuroanatomic atlases, are of significantly higher resolution than the PET images, again complicating interpretation in relation to the PET images.

In our initial image analysis we investigated three different paradigms in search of efficient and reliable anatomic mapping of uptake change. In the first paradigm, it was assumed that the anesthetized mice did not move between scan modalities and that the scanner manufacturer's automated geometric corrections were accurate. The CT images were then used to register the fed scan to the fasting scan and then to register the fused scans to the MRI atlas. The second paradigm allowed for the possibility that the mice had moved between modality scans or that the manufacturer's geometric corrections were incorrect, adding a PET-to-CT registration step (in both the fed and fasting images) before the other registrations. In the third paradigm, the fed and fasting PET images were registered directly, and the fused (averaged) PET image was registered to the MRI atlas. All registration was performed using the 3D normalized mutual information registration algorithm^[12] in Analyze^[13], evaluated and corrected manually from visual representation when necessary.

2.4 Scaling

The weight of each mouse and the activity of the prepared 2-deoxy-2-[^{18}F]fluoro-D-glucose was measured immediately prior to scanning. This allowed scaling the PET images into standard uptake values (SUV), which represent the absolute intensity of glucose metabolism in g/ml, a measure of brain activity at the time of the scan. This method allowed us to determine and analyze the absolute activation levels during the fasting (hunger/food anticipation) and fed (satiated) state. As discussed more fully below in the results section, we found that overall brain activity tended to be higher in the fasting state for all mice, albeit to a significantly different degree in the 3 experimental groups and in different brain regions. Therefore, in each case the fasting (baseline) PET images were subtracted from the fed images to create a map of uptake changes. This subtraction image could then also be visualized in relation to the MR image atlas or the neuroanatomic regions of the atlas, and the regional differences in uptake measured.

3. RESULTS

We found that the CT-to-CT registration was easily accomplished and easily evaluated. Even when significant time had passed between the fed and fasting scan, and when head position and body pose differed significantly between scans, the skull, soft tissue and air image provided a rich set of 3D structure to guide the registration algorithm and evaluate the quality of registration as shown in Figures 2 and 3. This step proved to be the most straightforward and repeatable of the registration functions, and the facility with which it was performed bodes well for the use of small-animal CT for longitudinal study.

Registration of the CT images to the MRI atlas data was less easily accomplished. This is to be expected, since the atlas data is an average of 12 individual 3D mouse brain images, and cannot be expected to fit exactly into the skull image of an individual mouse. Nonetheless, relative to the resolution of both modalities, one mouse is much like another, and it was often possible to automatically register the MR image data to the CT images. Evaluation was less straightforward than in the CT-to-CT case, and consisted mainly of evaluating how well the brain “fit” inside the skull as viewed in various cross-sections as shown in Figures 4 and 5. It was straightforward to detect serious misregistration, but very difficult to compare two candidate positions that were both near the correct answers, but noticeably different.

PET-to-CT image registration was similar to the CT-to-MRI registration in being less easy to automate or evaluate as shown in Figure 6. Since the PET images were of the same mouse as the CT image set, the “fit” within the skull was more exact, and somewhat easier to visualize. It may be more appropriate to use a surface based algorithm to register the PET brain “cloud” to the inside surface of the skull than to expect sparse voxel statistics to resolve the registration.

In an initial study performed in 3 AL mice we found that, despite the isoflurane anesthesia, the mice had indeed moved between the PET and CT scans. Attitudinal change of the head was visible in a comparison of the cross-modality scans of most mice at most time points. The first registration paradigm was consequently unsuccessful, and the subtracted PET images showed symmetric bilateral or anterior-posterior patterns related to the differences in pose between the PET and CT images at both time points, even though the fed CT to fasted CT registration seemed visually correct. A second set of mice (AL, LF and R3L-IGF-I-treated LF mice; n=3 each) were scanned under deeper anesthesia, and the misalignment between PET and CT poses was much reduced, though some independent motion of the individual mice between modalities was still observed. We were able to successfully register the fed CT image to the fasted CT using the Normalized Mutual Information-based 3D registration routine in Analyze™^[13] even though significant differences in whole-body pose were evident in most cases. The CT images offered enough contrast and detail that the automated algorithm’s results could be confidently assessed visually. We found it extremely difficult to visually evaluate the accuracy of PET-to-PET registrations. The first impediment is the low resolution of the PET images. There is a dim background signal from most of the brain, but this is a blurred and noisy “cloud” visually, and although the general shape allows gross alignment, several degrees of rotational misalignment are not visually obvious. Since the patterns of uptake were potentially different at the two time points, different regions of

the image “bloom” distorting the apparent shape between time points, confounding the registration algorithm and visual evaluation. Likewise, direct PET-to-atlas registration was very difficult to evaluate, and the third paradigm was abandoned.

In our second set of mice, using the second registration paradigm we found significant differences among the AL, LF and R3L-IGF-I-treated mice in global and regional glucose metabolism both in the fasted and fed state (Figure 7). After feeding, global cerebral hypometabolism (relative to the AL mice) was evident in both calorie-restricted groups irrespective of treatment (bottom row in Figure 7). This observation is consistent with reports of brain hypometabolism of glucose in patients suffering from anorexia or bulimia nervosa^[14, 15] and may reflect the effect of long-term caloric restriction. During fasting/food anticipation, only the R3L-IGF-I-treated LF mice, but not the LF mice, had reduced glucose metabolism relative to the AL group with significant differences in the pons and medulla, midbrain, pallidum, thalamus, internal capsule, basal forebrain, anterior commissure and the hippocampal fimbria (top row in Figure 7). Many of these regions have been associated with food intake^[16] and gastric sensations^[6, 17]. When fasted and fed states were compared, we found significant deactivation in response to feeding in most of these regions in the LF mice. While considerably less pronounced, the deactivation was also evident in the AL and R3L-IGF-I-treated mice. The overall pattern of deactivation by food intake in our mice appeared to be similar to what had been reported in lean humans^[16]. Thus, the degree of deactivation appears to distinguish untreated LF mice from the AL controls and the R3L-IGF-I-treated LF group in this pilot experiment. It is, however, important to point out that the detected differences may not be directly related to the effects of R3L-IGF-I on gastric muscles since in this set the untreated LF mice also had normal emptying of solids.

4. CONCLUSION

The combination of CT with PET for small animal imaging adds significantly to the potential of PET imaging for mapping uptake changes to specific neuroanatomic regions. Automated PET-to-CT and CT-to-CT registration of INVEON images is feasible and the results can be reliably evaluated visually. PET-to-PET registration of INVEON images is problematic, but can be achieved indirectly via PET-to-CT and CT-to-CT registration. INVEON CT mouse head image volumes can be convincingly registered to micro-MR image atlas. We expect that our approach will be amenable to comparing the feeding-associated central responses of mice exposed to chronic or chronic intermittent caloric restriction and in other mouse models of gastrointestinal motor dysfunctions.

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Figure 1.
Baseline PET image and atlas anatomic regions,

Figure 2.
Unregistered fed (left column) and fasting (right column) CT images.

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Figure 4.
MRI atlas in register with CT in the space of the atlas.

Figure 6.
Left panel – Pet (left column) and CT (right column) as imaged. Misregistration is most noticeable in the sagittal view; Right panel – PET/CT misregistration corrected.

Figure 7.
 Left column – *Ad Libitum* fed mice; Middle column – Limited Fed mice; Right column –
 Limited Fed + R3L-IGF-I; Top row – fasting; Bottom row – fed