**Reviewers' comments:**  
  
**Reviewer #1:**   
*Manuscript Summary:*   
As the authors indicate, accurate measurement of gastrointestinal (GI) motility is an important goal of many researchers who study GI function, and a JoVE publication the use of an approach that allow for recording of smooth muscle activity in vitro will be appealing. This is a very good example of a situation in which it will be much easier to learn and adapt the technique with access to a video that accompanies the written description of the preparation. A few queries and concerns are listed below.  
  
*Major Concerns:*  
I have no major concerns.  
  
*Minor Concerns:*  
1) When I was a postdoctoral fellow, my mentor read a draft manuscript that I had prepared, and suggested that I go on a "which hunt" whenever I write anything. It was a great lesson, and one that I still follow today. This manuscript includes an excessive, and often inappropriate, use of the term "which". I suggest that the authors go on a "which hunt". Also, I suggest that they refer to the following web site, which includes a nice description of how to use this term properly. <http://www.montana.edu/gradwriting/?p=15>

Correction made as requested.  
  
2) I was somewhat surprised by the use of "gage" rather than "gauge" in this manuscript. While "gage" is not wrong, "gauge" is a much more common spelling for this use. Also, the authors slip and use the "gauge" spelling in at least 4 instances throughout the manuscript.

Gage is the proper spelling used by suppliers of these and similar components. We chose to use the format whereby gage is an instrument used to measure and gauge is a measurement standard or scale. We misspelled strain gage twice as “strain gauge” (reflecting our past tendency to use gauge). These mistakes were fixed. However, the spelling “gauge” was retained for the other uses of the word: 25 gauge needle and fine gauge wire  
  
3) Last sentence of the Abstract. "Data" is a plural term, and "included including" is awkward. Please rephrase to "…data from the entire GI tract are included, along with discussion…"

This change was made  
  
4) Section 1.3.4 (lines 178 - 180). Add a reference to Fig 1E.

The figure reference was added  
  
5) Line 208. Insert a space in "amplifierusing".

A space was inserted to separate “amplifier” and “using.”  
  
6) Line 212. Change to "Adjust the Brindge, Balance, and Gain settings…"

This change was made  
  
7) Sents on lines 233-234. This sentence makes no sense. The angle of the mandible is the junction of the body and the ramus, and the sternal notch is a midline structure, so you cannot get more proximal than that. Suggested revision: "Make a 1-2 inch midline incision from the inferior border of mandible to the sternal notch.

This sentence was intended to suggest direction during the incision. Since the direction of the cut is irrelevant the sentence was revised as suggested. We also caught the erroneous use of inch rather than cm.

8) Line 261. Change "terminal" to rostral or caudal, whichever you are trying to describe.

“Terminal” was changed to caudal.  
  
9) Line 272. Change "…in order to produce the best data" to "to obtain the best results".

This line was changed.  
  
10) Lines 283-284. How will you maintain temperature? A heating blanket?

A feedback controlled heating pad is used to maintain temperature, and this was added for clarification  
  
11) Line 302. Change "is" to "are"

This change was made  
  
12) Line 371. Change "an individual can easily run up to four animals" to "a single investigator can acquire data from up to for animals".

This change was made  
  
  
**Reviewer #2:**   
The paper titled "Fabrication and implantation of miniature dual-element strain gages for measuring in vivo gastrointestinal contractions in rodents" by Holmes et al. provides a step-by-step guide to the fabrication of the current design of strain gages for measuring gastric motility in rats. The paper describes a clear methodology for measuring gastric contractions in rats; however, the measurement of contractions in the gastrointestinal tract with a strain gauge transducer, as described in this paper, does not include any novel, special techniques or knowledge.

In the manuscript, the authors measured gastric contractions in an anesthetic state; however, the measurement of gastric contractions in the conscious state is very important. In fact, strain gauge transducers were sutured on the stomachs of rats and gastric contractions were recorded in the conscious state (Ariga, H et al, Regul Pept 2008;146:112-116; Taniguchi, H et al, Am J Physiol Gastrointest Liver Physiol 2008;295:G403-411).

We are quite familiar with the strain gage used by the Takahashi group (and they have provided us with several gages of their own fabrication). These gages are of different materials and design and are dissimilar from what we describe. To our knowledge, the fabrication of the Takahashi group design has not been described fully. Fabricating the gages is the primary point of this manuscript; application is more for the reader to determine.

Furthermore, in suncus murinus, known as a small animal model (smaller than rats) of gastrointestinal motility, the same techniques were performed and the gastric and duodenal contractions were measured at the same time in the conscious state (Sakahara, S et al, Am J Physiol Regul Integr Comp Physiol 2010;299:R1106-1113).

As best as can be discerned from tracing the literature for the Sakai group and the Japanese web pages where materials for these strain gages were acquired, we wish to point out that the strain gage used in the Sakahara paper is lower resistance and therefore will be more prone to electrical noise. Furthermore, the Beryllium-copper backing element (though malleable for some applications) would be prone to damage (bending, etc.) in this biological application. We also wish to emphasize that the description regarding the fabrication of this strain gage is incomplete.

With particular emphasis to our manuscript, we make no assertion that this application of gages is proprietary. It is simply a matter of fact that laboratories now need to fabricate their own gages and that this process requires certain skill sets that are best presented in the unique JOVE format. Similar occurrences for other techniques have previously appeared in JOVE publications, and this led us to decide to select JOVE. Please see our original point in the discussion (with particular emphasis on the last sentence):

Since the commercial manufacture of these strain gages has ceased, laboratories investigating gastrointestinal function are limited to other techniques which may not permit the full range of experimental applications that are available. This report provides an updated and more detailed description of previously described techniques 13. The text and accompanying video specifically address solutions to common pitfalls that we recognized during development and mastery of the fabrication process.

Thus, the advantages of this paper are not clear and it is currently difficult to accept this manuscript.

Special techniques and knowledge are required and our main point regarding the advantage of this paper rests with the monetary and time losses accrued over the number of strain gages we wasted learning the necessary fabrication skills *de novo*. I feel that it is important to preserve the knowledge base for constructing these gages for future researchers.

In addition to the major concerns mentioned above, several comments that the authors need to consider to improve their manuscript are described below.  
  
Other issues:  
1. The measurements of normal gastric motor patterns during the fasting and postprandial states obtained by using your method should be mentioned.

We respectfully submit that the purpose of measuring contractions in this manuscript was to validate the output of the strain gage that was fabricated and pictured in the manuscript. Discussion of motor patterns during fasting and postprandial states would require additional background information and is perhaps more appropriately saved for quantitative reports using the gages.  
  
2. In what stage/phase of the gastric motor pattern did you administer TRH and SNP to show the rise/inhibition of the contraction?

We routinely monitor the gastric contractions for 30 min to ensure a stable baseline prior to any pharmacological manipulation. Again, we respectfully suggest that the presentation and discussion regarding the phase of the gastric motor pattern is inappropriate given the wealth of scientific literature in which these two agents have been applied in studies of GI physiology. They were employed as pharmacological tools for validation only.  
  
3. Did you check the sensitivity of the strain gage after the preparation/before implantation? This should be described in the results.

This was described in section 1.5.3  
  
4. In line 213, please specify the bridge amplifier reference and also clarify the bridge and balance settings on the strain gauge amplifier.

Such a description would be specific to only one amplifier out of many available commercially. The aim of the paper was the fabrication and representative validation of a strain gage. To try to instruct users on using basic instrumentation would exceed the scope of the paper and would replicate information available in the user manual.  
  
5. How was brainstem administration of TRH and intravenous administration of SNP provided? Detailed administration methods need to be included.

Additional descriptive text has been provided as requested (Section 3).

**Reviewer #3:**   
*Manuscript Summary:*   
- The skill of preparing and the use of the proposed strain-gage is very useful tool in gut motility studies and is by itself valuable contribution. The dual-element strain-gage involves abdominal surgery, implantation of the transducer and tunnelization and securing of the free ends, similar to EMG. This, unlike manometry, is a drawback. However strain-gage has a better spatial resolution than manometry, although side hole manometry has a better spatial resolution than the traditional manometry.

We also wish to point out that the orogastric route utilized in the manometric technique has similar pitfalls to the non-invasive pressure transducer we discuss in the introduction. Namely, that it is inherently limited to anesthetized animals as well as the spatial resolution problems mentioned above.  
  
Major comments  
- The proposed strain-gage, although is mini sized compared to previous generation of strain-gages, has a finished product size of 6X8 mm. This size while very useful for stomach and larger organs, it will be a limitation particularly for mice and discrete gut regions such as sphincters and narrow regions such as esophagus. This needs to be outlined.

We would like to ask Reviewer #3 to cross reference our response to this concern with that of Reviewer #2 (main paragraph). Reviewer #2 referred to a 2010 paper by Sakahara et al., which reported strain gage recordings from the musk shrew (approximately 50% the size of the smallest rats we utilize). While the authors only described fabrication of the chronic strain gage as a modification of their “own design” we located another paper by the same group (Miyano 2013, PLOS1) which listed the dimensions as 7.5X7.0 mm. Clearly there is considerable latitude regarding the size limitations.

In an effort to stay within the 3-6 paragraph limitation for the discussion, we have modified the 4th paragraph (underlined) of our discussion to state:

Encapsulating the dual element in three layers of silicone creates a durable and flexible yet highly sensitive strain gage that will last over repeated use with proper care. The high sensitivity of an unencapsulated strain gage is minimally affected by any resistance that is imparted by the silicon laminate. Thinner silicone sheets (P/N 20-05) are recommended in order to modify the gage for intestinal applications or for fabricating smaller gages for mice and discrete gut regions such as sphincters and esophagus. Extra caution is required since thinner gages have diminished resistance to tearing of the silicone sheet during implantation.

- It is to be noted also that strain-gage is a force transducer and as such the data generated is not a direct motion data but rather a concomitant of motion. This is true for most of the methods used. EMG and manometry use electrical and pressure signals to make sense of motion. The silicon casing in which the strain-gage is embedded imparts a level of resistance to the force of contraction. Thus the temporal resolution, while acceptable, could be less than that of EMG or sonomicrometry. This need to be discussed.

We fully agree with the reviewer’s first observation on concomitant of motion. We wish to point out that the high sensitivity of an unencapsulated strain gage is minimally affected by any reduction in sensitivity that is introduced by the silicon laminate.

See our addition to the discussion listed above.

Since gastric muscle contraction waves are not a particularly high-speed event (except, perhaps, during emesis) and occur at a rate of ca. 5 cycles/min the temporal advantages of sonomicrometry may be unnecessary.

Furthermore, our (GMH) past experience with sonomicrometry systems (albeit ca. 2004, when first published) was disappointing due to the overriding respiratory artifact and poor signal resolution coupled with the potential for an enormous volume of data that must be processed. Recall that circular \*and\* longitudinal contraction forces occur in the stomach and were targeted by Adelson et al, (Am J Physiol-Gastro, 2860:G321-G332, 2004). We would argue (and we recognize others would disagree) that a minimum of a 2X2 array of crystals (generating 12 individual distances to be compared) is necessary for convincing data for each region being investigated. Xue et al. (Am J Physiol-Gastro, 290:G74-G82, 2006) delineated the potential for erroneous conclusions (when one region affects movement within another) with this technique in their discussion. The apparently few GI-related publications (based on a literature and citation search) using this technique may indicate that others have come to similar conclusions. We did not fully introduce this concern in the manuscript due to space limitations and the fact that a full indictment of any given technique isn’t the intended topic of the paper. All techniques have strengths and weaknesses.

We have added the following to the introduction:

Ultrasonomicrometry has been employed in some GI studies (Adelson et al., 2004; Xue et al., 2006) in order to take advantage of the small size, spatial, and temporal advantages of piezoelectric crystal transmitter/receivers. Waves of gastric smooth muscle contraction are not a high-frequency event and occur at a rate of approximately 3-5 cycles/min. Therefore, the temporal advantages of sonomicrometry may be unnecessary to justify the cost. Furthermore, limitations have been presented regarding accurate data interpretation that may result from implanting an insufficient number of crystals (Xue et al., 2006).

- There is no enough information on the instrumentation of strain gage (section 2.3). It is not clear how the 4-5 cm silk threading of the four corners of the gage is to be used in the implantation process.

This was one step that we feel will be best visualized through the video. For clarity, we have made the following change:

2.3.4) Align the grid of the encapsulated strain gage in parallel with the circular smooth muscle fibers. Using the previously threaded sutures (step 2.3.1), attach the corners of the gage to the ventral serosal surface of the gastric corpus using a #14 taper point 3/8 circle needle. In order to minimize tissue damage and potential bleeding, do not use cutting-edged needles and do not perforate any superficial blood vessels on the surface of the stomach.

- Silk is the thread that is suggested however silk is not recommended for survival surgery and this has to be emphasized and other alternatives proposed.

Silk thread is acceptable for non-survival surgeries and for internal applications, where the wicking of bacteria across an epithelial barrier is not a risk. As we addressed for other comments regarding chronic applications, full and accurate discussion of chronic procedures greatly exceed the focus of the manuscript.   
  
- Exteriorization of the leads is not well explained. If it requires subcutaneous tunnelization, it should be stated. If ileal strain gage are exteriorized through the abdominal incision, how are they protected from being damaged by the rat?

Subcutaneous tunneling would only be required for chronic implantation. There is not risk to the wire in an anesthetized rat. While we can’t succinctly address alternative approaches for chronic implantation, we have made the following changes:

2.3.7) Exteriorize the strain gage leads through the wound margin before closure of the abdominal incision. Secure the free wires to the animal (eg., hind foot) in order to provide strain relief during manipulation of the animal or terminal wire connector. Close the rectus abdominus muscles and the abdominal skin separately with 3-0 nylon suture. In a chronic model, secure the leads subcutaneously along the dorsal side of the rat and exteriorize them above the skull 18.

Perhaps the editor could address the suitability of inserting the following in the discussion or as an endnote:

For illustrative purposes, our protocol only describes implantation in a terminal preparation. The procedures for chronic implantation require attaching the wire leads to a headstage pedestal affixed to the skull. These procedures have been described elsewhere (Miyano et al., 2013) and a careful review of such techniques is beyond the scope of this paper.

- Examples of use are mentioned only for circular muscle contraction study. What is the fidelity and validity of strain-gage use for longitudinal muscle in vivo measurement?

Since the sensitivity of the gage is along one axis, rotating the gage 90 degrees would detect longitudinal contractions.  
  
- The use for chronic experiments is not well validated.

The reason text detailing chronic experiments was not included is discussed above

Minor comments   
- The paper should acknowledge ultrasonomicrometry as a tool to measure the actual gut motion, not concomitant.

Discussed above