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Fabrication and implantation of miniature dual-element strain gages for measuring in vivo gastrointestinal contractions in rodents.

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Abstract:	Gastrointestinal dysfunction remains a major cause of morbidity and mortality. Indeed, gastrointestinal (GI) motility in health and disease remains an area of productive research with over 1,400 published animal studies in just the last 5 years. Numerous techniques have been developed for quantifying smooth muscle activity of the stomach, small intestine, and colon. In vitro and ex vivo techniques offer powerful tools for mechanistic studies of GI function, but outside the context of the integrated systems inherent to an intact organism. Typically, measuring in vivo smooth muscle contractions of the stomach has involved an anesthetized preparation coupled with the introduction of a surgically placed pressure sensor, a static pressure load such as a mildly inflated balloon or by distending the stomach with fluid under barostatically-controlled feedback. Yet many of these approaches present unique disadvantages regarding both the interpretation of results as well as applicability for in vivo use in conscious experimental animal models. The use of dual element strain gages that have been affixed to the serosal surface of the GI tract has offered numerous experimental advantages which may continue to outweigh the disadvantages. Since these gages are not commercially available, this video presentation provides a detailed, step-by-step guide to the fabrication of the current design of these gages. While we use our design version for recording gastric motility in rats as an example, this overall design has been tailored for recording smooth muscle activity along the entire GI tract and requires only subtle variation in the overall fabrication. Representative data from the entire GI tract are included as well as discussion of analysis methods, data interpretation and presentation.
Author Comments:	As I described in my original cover letter, this manuscript takes advantage of the unique features of JOVE to demonstrate the fabrication of strain gages for smooth muscle contractions. As we document in the text, these strain gages are based upon

	the work of the late Dr. Paul Bass and colleagues. While the original description of the construction provides a starting point for manufacturing these strain gages, some of the protocol details that I expect to feature during videotaping center on the little tricks that I had to learn by trial and error. I wasted quite a few strain gage components during the trial and error phase of making my own gages. Due to my own challenges in making these gages, I feel that it is important to preserve the knowledge base for constructing these gages for future researchers. As I propose in the text; there are several advantages to these strain gages that have yet to be matched by other techniques.
Additional Information:	
Question	**Response**

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Dear Colleague,

I wish to submit for consideration our revised manuscript entitled "Fabrication and implantation of miniature dual-element strain gages for measuring *in vivo* gastrointestinal contractions in rodents." for review and publication in *Journal of Visualized Experiments*. Our revisions of concerns raised by the peer reviewers are documented in our tracked changes version of the manuscript and our point by point response to the comments (separate file, our text is in red).

As we stated previously, this work seeks to take advantage of the unique visual format of JOVE to demonstrate some of the finer details of fabricating strain gages for smooth muscle contractions. As we note in the text, this design (based upon the original work of the late Dr. Paul Bass) is no longer available. RB Electronics was a small business enterprise that would fabricate these strain gages based upon Dr. Bass' design, but ceased production in 2010. I feel that it is important to document this fabrication process for posterity. Some of the protocol details that I expect to feature during videotaping center on the little tricks that I had to learn by trial and error. I would have benefited greatly by knowing these details and certainly not wasted time, effort and materials in the process.

At the early stage of writing this manuscript I tried to contact Dr. Bass to invite him to collaborate. Sadly, I learned that he had passed away some weeks prior to my phone call. As a result of these events, I believe that there are no intellectual or commercial conflicts that would preclude describing the expanded protocol for fabrication in JOVE.

Emily Swartz, Margaret McLean and myself have not submitted this manuscript elsewhere, and have no conflicts of interest associated with publication of this protocol.

We appreciate the efforts of you and your staff in the review process and we look forward to hearing from you.

Sincerely,

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Fabrication and implantation of miniature dual-element strain gages for measuring *in vivo* gastrointestinal contractions in rodents.

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Short Abstract

The *in vivo* measurement of smooth muscle contractions along the gastrointestinal tract of laboratory animals remains a powerful, though underutilized, technique. Flexible, dual element strain gages are not commercially available and require fabrication. This protocol describes the construction of reliable, inexpensive strain gages for acute or chronic implantation in rodents.

Long Abstract

Gastrointestinal dysfunction remains a major cause of morbidity and mortality. Indeed, gastrointestinal (GI) motility in health and disease remains an area of productive research with over 1,400 published animal studies in just the last 5 years. Numerous techniques have been developed for quantifying smooth muscle activity of the stomach, small intestine, and colon. *In vitro* and *ex vivo* techniques offer powerful tools for mechanistic studies of GI function, but outside the context of the integrated systems inherent to an intact organism. Typically, measuring *in vivo* smooth muscle contractions of the stomach has involved an anesthetized preparation coupled with the introduction of a surgically placed pressure sensor, a static pressure load such as a mildly inflated balloon or by distending the stomach with fluid under barostatically-controlled feedback. Yet many of these approaches present unique disadvantages regarding both the interpretation of results as well as applicability for *in vivo* use in conscious experimental animal models. The use of dual element strain gages that have been affixed to the serosal surface of the GI tract has offered numerous experimental advantages, which may continue to outweigh the disadvantages. Since these gages are not commercially available, this video presentation provides a detailed, step-by-step guide to the fabrication of the current design of these gages. The strain gage described in this protocol is a design for recording gastric motility in rats. This design has been modified for recording smooth muscle activity along the entire GI tract and requires only subtle variation in the overall fabrication. Representative data from the entire GI tract are included as well as discussion of analysis methods, data interpretation and presentation.

Introduction

Experimental studies that record *in vivo* gastrointestinal (GI) motility across a number of experimental conditions remain a powerful tool for understanding the underlying normal and pathophysiological processes necessary for nutrient homeostasis. Traditionally, numerous experimental methodologies, some with similarities to those found in clinical practice [1], have been employed to directly quantify changes in GI contraction rate [2-5], intraluminal pressure [6, 7], or the GI transit of non-absorbable markers [8, 9] or stable isotopes [10-12]. Each of these techniques has unique advantages and disadvantages, which have been addressed previously in the literature. For example, the utility of balloon manometry to quantify pressure changes has been questioned due to the inherent compliance of the balloon material while gastrointestinal recovery of non-absorbable markers requires euthanizing the experimental animal for a single data point. Recently, the application and validation of a miniaturized arterial pressure catheter has been reported that offers a non-surgical method for monitoring gastric contractility in rats and mice [3]. While an orogastrically placed pressure transducer effectively eliminates confounding variables on gastrointestinal function by avoiding invasive surgical procedures, such an approach is only suitable for anesthetized preparations. Furthermore, the lack of visual guidance does not permit consistent placement of the transducer within specific regions of the stomach. As such, this application is restricted to the stomach or colon since visualization, coupled with the relatively stiff transducer wire, within the duodenum or ileum is not an option.

Similarly, the bio-magnetic alternate current biosusceptometry (ACB) technique has been validated for GI contraction analysis[4]. While the ACB technique provides a non-invasive approach for measuring gastrointestinal contractions, ACB suffers from a similar limitation in that the use of ingested magnetic detection media does not permit precise recording of specific regions of the GI tract. This limitation can be overcome through the surgical implantation of magnetic markers. Nonetheless, the ACB technique necessitates that the animal be anesthetized for data collection.

Ultrasonomicrometry has been employed in some GI studies 13, 14 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, while linear motion is accurately measured with sonomicrometry, limitations have been presented regarding accurate gastrointestinal data interpretation that may result from implanting an insufficient number of crystals14.

Based upon the original designs of Bass and colleagues [2, 15] this visualized protocol more fully documents the step-by-step fabrication and experimental application of miniature, dual element strain gages that possess high sensitivity and flexibility for recording smooth muscle contractions along the entire GI tract. The dimensions of the strain gage elements are suitable for any rodent application since sensitivity and size of

the finished strain gage are most dependent upon the silicone sheets encapsulating the elements. These strain gages are readily adapted for acute and chronic application in anesthetized and freely behaving laboratory animal models thereby providing a single technique for quantifying smooth muscle contractions.

PROTOCOL

All procedures followed National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee at the Penn State Hershey College of Medicine. Rats were housed using common vivarium practices. Note: This protocol uses male Wistar rats ≥8 weeks of age and initially weighing 175-200 g.

1. Procedures for fabrication of strain gage

Most tooling and components remain available from the original or successor companies and are summarized in Table 1.

1.1) Preparation and Bonding of two single element strain gages

1.1.1) Handle the strain gage elements (EA-06-031-350) carefully with clean Dumont #5 forceps. To limit unwanted movement of elements, use a small, clean, self-adhesive piece of paper with the adhesive side facing up to secure elements to the work surface without the risk of contamination or excessive adhesion.

1.1.2) Bond two single strain gage elements back-to-back, to form a dual element. Clean the back of each element film with Isopropyl alcohol and allow drying by evaporation (drying with gauze often introduces fiber contaminants that are difficult to remove). Under stereomicroscope guidance (1-3X), and using a clean artist brush (10-0 camel hair), apply a thin film of epoxy-phenolic adhesive to the back of one element and immediately place the opposing back of the second element in contact and align the foil grids. (Fig. 1A).

1.1.2) Place the bonded elements in a 50-60°C oven overnight to fully cure the epoxy.

Note: Do not clamp the bonded elements since excess epoxy may seep onto the elements and pressure may cause misalignment of the grids. The two-part epoxy has a usable refrigerated-life of only 6 weeks after mixing. Bond and cure a sufficient supply of elements at one time and store them in a clean, dust-free, environment for later use.

1.2) Sizing and wiring dual element strain gages

1.2.1) Trim bonded dual elements to a final size of 3X3mm with a #11 scalpel blade. Delay trimming the topmost portion of the dual elements at this time in order to have an area for safely handling the element. (Fig 1A).

1.2.2) Each element requires a four-conductor wire fabricated from three-conductor, bondable, Teflon insulated wire (P/N 336-FTE). Disassemble one 30cm braided strand of three-conductor wire into three constituent wires

1.2.3) To make a four wire cable, pair one of the resulting single wires with the like-colored wire contained within a second 30cm length of three-conductor wire. In the following steps, these matching colored wires will be joined at the terminal end to form a common wire for the final strain gage (Fig 1B).

1.2.4) Remove approximately 1mm of Teflon insulation from both ends of each wire with thermal wire strippers . Using activated rosin soldering flux and low temperature solder (melting point 183º C) tin the wire ends with a soldering pencil.

1.2.5) For the next stage, a micro-soldering tip is needed to form a more discrete solder joint to prevent heat damage to the film layer of the element (Fig. 1C). To fabricate a smaller micro-soldering tip, wrap a small piece of copper wire (~0.25mm diameter) once around the standard soldering tip, ensuring that the copper wire extends beyond the length of the standard soldering tip.

1.2.6) Flux just the solder pads on one side of the bonded dual element with a clean 10-0 brush and solder one single lead and one of the paired common leads to the solder pad (Fig. 1D). Residual flux can be removed afterward with a clean brush dipped in resin solvent

1.2.7) Repeat the process on the opposite side, ensuring that the remaining common wire lead is soldered to the pad opposite the original common lead.

1.3 Testing and epoxying dual element strain gages

1.3.1) Solder gold socket connectors (E363/0) to the free ends of the wire leads. At this point, connect the strain gage to a recording amplifier (described below) to test the integrity of the dual element assembly.

1.3.2) Measure the resistance with a good-quality volt-ohm meter. Elements register a resistance of approximately 350 Ω. Re-solder inadequate connections at this point with fresh solder.

1.3.3) If the solder connections and the dual element assembly are deemed satisfactory, trim off any remaining element film.

1.3.4) Insulate the solder joints on the element solder pads with a thin layer of two-part silicone-rubber epoxy resin (P/N E211). For best results, partly cure the resin for 20-30 min prior to application (Fig 1E).

1.4) Encapsulating dual element strain gages in silicone

1.4.1) Cut three pieces of 0.5mm thick silicone sheet (P/N 20-20) to 15mm^2 and clean the silicone with distilled water. Cut one piece of silicone sheet into a U-shape in order to accommodate the final dual element assembly without deforming the encapsulating silicone (Fig 1F).

1.4.2) Coat the inner surfaces of the notch-free silicone sheets with clear silicone adhesive.

1.4.3) Sandwich the dual element assembly within the notch and the aligned outer sheets, then gently press out any excess silicone, as well as air bubbles, from the center outward. Carefully clamp the encapsulated assembly between two blocks of metal bar stock for 24 hr to ensure uniform thickness and that no deformations occur.

1.4.4) Allow the excess silicone to remain along the boundaries of the assembly and cure. This excess will be removed when the sheet silicone is trimmed to the desired final dimensions (commonly 6mmX8mm; Fig. 1G).

1.5) Completion of wire connector and calibration

1.5.1) Reinforce the solder joint of the gold socket connectors on the individual terminal wire leads with 3mm (1/8 inch) shrink tubing and align within a plastic electrode pedestal (MS363, Fig. 1H). Secure the electrode pedestal and wires with 0.125- and 0.25-inch diameter shrink tubing to prevent disconnection during the experiment (Fig. 1I).

1.5.2) Strain gage signals are processed through a high gain bridge amplifier (P/NAMP-01-SG). Connect the strain gage to the amplifier using a cable with a mated plug (363-SL/6) to match the electrode pedestal. The threaded cap provides additional security to maintain uninterrupted signals during the experiment.

1.5.3) Adjust the Bridge, Balance and Gain settings on the amplifier to a dedicated strain gage per manufacturer instructions. Affix the end of the strain gage where the wires exit horizontally to a rigid clamp and calibrate by placing a 1g static load on the opposite end as originally described by Pascaud and colleagues[2, 16, 17].

2. Surgical Procedures for Acute Implantation of Strain Gage

2.1) Animal Care and Preparation:

2.1.1) Food deprive experimental animals the night before surgical implantation (water may be provided ad libitum).

2.1.2) Deeply anesthetize the animal. Thiobutabarbital (100–150 mg/kg; i.p. for rats) is preferred for terminal (ie., non-survival) strain gage implantation and experimentation due to sustained anesthetic effect and minimal alteration of gastric reflexes in the rat [10]. Test for absence of paw pinch reflex to determine depth of anesthesia.

2.1.3) Prepare the rat for aseptic surgery as dictated by the experimental design and approved IACUC guidelines including sterilizing surgical tools, shaving incision sites, applying vet eye ointment and disinfecting all surgical areas.

2.2) Tracheal intubation for terminal experiments:

2.2.1) For long-duration, terminal, experiments the intubate the rat with a tracheal tube to maintain an open airway. Make a 1-2-cm midline incision on the ventral side of the neck from the inferior border of the mandible to the sternal notch.

2.2.2) Separate the underlying strap muscles using blunt dissection at the midline to expose the trachea. Isolate the trachea from the underlying esophagus and place a loop of 3-0 ethilon suture between the trachea and esophagus to form a ligature.

2.2.3) Open the trachea anteriorly by making a small cut in the membrane between two of the cartilaginous rings of the trachea just distal to the thyroid gland. Insert a small piece of polyethylene tubing (P/N PE-270), 5mm in length and beveled at one end) into the trachea and secure it into place with the ligature.

2.2.4) Put the strap muscles back in place and suture the overlying skin with 3-0 ethilon.

2.3) Strain gage instrumentation to gastrointestinal surface:

2.3.1) Thread the four corners of the strain gage with 4-5cm lengths of 4-0, or smaller, sterile silk suture using a #14 taper point 3/8 circle needle prior to surgery. Silk suture provides a high level of flexibility and is less likely to damage the silicone encapsulating the strain gage element.

Note that 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. In applications requiring survival surgery, a prolene suture is necessary in order to reduce the risk of infection inherent in the braided cloth fibers of the silk suture.

2.3.2) Perform a laparotomy by incising the abdominal skin along the midline. Section the rectus abdominus musculature along the connecting linea alba (avascular) to prevent bleeding. Then make a very superficial midline incision in the parietal peritoneum to avoid lacerating underlying abdominal viscera.

2.3.3) Exteriorize the stomach with the aid of saline-soaked cotton tipped applicators. Keep the stomach in its anatomical position by carefully placing it on a saline-soaked gauze pad at the caudal end of the abdominal incision.

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.

2.3.5) Begin the suture pattern of the gage along the greater curvature of the stomach near the fundus/corpus boundary and proceed next along the fundus/corpus boundary toward the lesser curvature. The serosa underlying the strain gage should neither be slack nor overly stretched in order to obtain the best results.

2.3.6) Carefully return the stomach to its anatomical position using saline-soaked cotton tipped applicators.

2.3.7) In an acute model, exteriorize the strain gage leads at the caudal end of the midline incision 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].

2.3.8) After surgical instrumentation, place animals in a stereotaxic frame to support the head and elevate the upper torso. The latter step helps to reduce respiration artifact during recording. Monitor rectal temperature and maintain at 37±1°C using a feedback-controlled heating pad.

2.3.9) At the conclusion of terminal experiments utilizing thiobutabarbital anesthesia, the animal must be euthanized in a manner consistent with American Veterinary Medical Association (AVMA) Guidelines on Euthanasia.

2.4) **Gastric Motility recordings:**

2.4.1) Amplify the strain gage signal with any commercially available DC bridge amplifier.

2.4.2) Record the DC output signals on a computer using the chart recorder function of any commercially available data acquisition system.

Note: A hardcopy of the amplifier output can be generated through a polygraph chart recorder.

3. Representative Measurement of Gastric Contractions Following Brainstem Stimulation

3.1 Exposure of brainstem and fourth ventricle

3.1.1) After surgical instrumentation, and placement of the Thiobutabarbital-anesthetized animal in a stereotaxic frame, make a 1.5-2 cm midline skin incision from the occipital bone toward the base of the neck.

3.1.2) Separate the connective tissue joining the bilateral muscle bellies of the underlying neck muscles along the midline (muscles from superficial to deep are levator outis longus cranial portion, levator outis longus caudal portion, and platysma cranial portion).

3.1.3) Detach the levator outis longus from the occipital bone once the midline is clearly defined and exposed.

3.1.4) Carefully expose the caudal region of the skull by using blunt dissection to detach platysma muscle from the underlying dura mater.

3.1.5) Use a new 25 gauge needle to carefully detach the dura mater along the foramen magnum extending bilaterally to the occipital condyles.

3.1.6) Use #5 Dumont forceps to remove the pia and arachnoid meninges overlying the fourth ventricle and expose the brainstem.

3.2 Administering fourth ventricle thyrotropin releasing hormone or intravenous sodium nitroprusside

3.2.1) Weigh and dissolve thyrotropin releasing hormone (TRH) in sterile saline to reach a final concentration of 5 μM TRH.

3.2.2) Weigh and dissolve sodium nitroprusside (SNP) in sterile saline to reach a final concentration of 150 μM SNP.

3.2.3) Using a 10 μl syringe, administer 2 μl of TRH (final dose equals 100 pmol) to the dorsal surface of the brainstem fourth ventricle to facilitate recording of gastric contractions.

3.2.4) Using a sterile syringe and 27 gauge needle, administer 150 μmol/kg of SNP through the tail vein to facilitate recording of gastric relaxation.

REPRESENTATIVE RESULTS

Representative data from a Thiobutabarbital -anesthetized rat are shown in Figure 2. The top trace represents the gastric corpus contractions from the rat during the brainstem administration of thyrotropin releasing hormone (TRH, 100 pmol), a known motility-enhancing peptide[3, 19]. It shows baseline contractions prior to the increase in phasic gastric smooth muscle activity. Note: Analysis of these peaks in gastric contractions follow the original formula devised by Ormsby and Bass[20]

$$\text{Motility Index} = (N_1 x1) + (N_2 x2) + (N_3 x4) + (N_4 x8)$$

Based upon this formula, N equals the total number of peaks in a particular milligram range. Therefore, presuming that a 0 mg signal is indicative of no gastric motility, the grouping of peak-to-peak sinusoidal signals may be calculated as 25-50 mg, 60-100 mg, 110-200 mg and signals greater than 210 mg for N_1 through N_4, respectively. This formula is less sensitive to baseline tone fluctuations that naturally occur across several seconds or minutes. Such fluctuations would have to be subtracted in order to generate valid area using under the curve measurements[3].

The second trace demonstrates a reduction in baseline gastric smooth muscle tone from the same animal in response to the nitric oxide donor, sodium nitroprusside (20mg/kg iv). Data representing an inhibition of gastric smooth muscle activity are readily analyzed by the reduction in signal voltage between baseline and maximal response. This voltage signal can then be used to derive the equivalent static load, in grams, if the strain gage was calibrated prior to the experiment. These representative data demonstrate the bidirectional capabilities of a dual element strain gage that has been properly attached to the gastric serosa.

The third trace represents basal smooth muscle contractions recorded by a sub-miniature strain gage sutured to the serosal surface of the duodenum of a fasted rat. The orientation of the strain gage elements were also in parallel with the circular muscle of the duodenum.

Figure legends

Figure 1. Principal stages of strain gage fabrication. A) Dual bonded elements that have been trimmed on three of four sides to final dimensions. B) Representative ends of wires configured for attachment to gage elements (left) and terminal connectors (right). Note that dual read leads are joined only at the terminal end (arrowhead). C) Representative placement of a strand of copper wire in proximity to fine (1.5mm) soldering tip. Maintaining fresh solder along this junction (arrowhead) ensures sufficient heat transfer through the micro tip to melt 63% Tin:36.65% Lead:0.35% Antimony solder. D) Representative extent of solder joints between wire leads and solder pads on the gage element. E) Properly potted solder joints. F) Representative notch in the internal silicone laminate sheet to accommodate strain gage element without deforming completed element. G) Bonded layers of silicone sheets (three in total) forming a completed strain gage prior to final sizing. H) Wire connections to gold plated sockets

are reinforced with layers of succeedingly larger diameter shrink tubing before insertion into electrode pedestal. I) Final shrink wrap affixing of terminal connectors and electrode pedestal. Calibration bars: A-D, 5mm; E, 2mm; & F-I, 5mm.

Figure 2. Representative motility traces generated with fabricated dual element strain gages. Recordings made from the anterior gastric corpus during an increase in gastric contractions (top trace) and during an inhibition of gastric contractions (middle trace) and duodenum (bottom trace) of fasted rats (200-250g).

DISCUSSION

The procedures presented here allow individual laboratories to fabricate sensitive miniature strain gages for biological applications including, but not limited to, gastrointestinal motility in small laboratory animals. 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 [15]. The text and accompanying video specifically address solutions to common pitfalls that we recognized during development and mastery of the fabrication process.

Each step, as described, presents techniques to successful fabrication. Careful attention to cleanly and securely soldering all connections as well as avoiding damage to the element with excessive heat from the soldering process are the most frequent challenges to success. The fine gauge wire is prone to breaking if it is not properly reinforced with shrink tubing or silicone epoxy and will result in an absence of signal when the gage is gently flexed. A strain gage with a broken or disconnected wire in the vicinity of the gold connectors within the plastic terminal pedestal is the most common failure of a previously functional gage. Individual gages can be carefully disassembled by removing the shrink tubing in order to expose the broken wire. After re-soldering the wire to the gold connector, the entire gage is reassembled with new shrink tubing.

With a bit of practice and careful attention to fabricating strain gages of uniform dimensions, affixing strain gages relative to clear landmarks (eg. Greater gastric curvature, fundus/corpus boundary), and avoiding damage to the vasculature, novice users will rapidly develop the ability to achieve consistent results.

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.

Surgical difficulties with the use of these gages often result from excessive manipulation of the visceral organs or misalignment of the gage during implantation. The former likely initiates neural and inflammatory processes that directly lead to impaired GI motility, 9, 21 though both pitfalls are easily remedied by refinement of surgical technique. This may include altering the length and starting point of the midline incision into the abdomen as well as minimizing the manipulation of the viscera during exteriorization and replacement of the stomach.

The validity and fidelity of these strain gages have been discussed previously [2, 15]. We, and others, routinely measure gastric smooth muscle activity in acute, anesthetized preparations [16, 22] . With adequate instrumentation, a single investigator can instrument and acquire data from up to four animals in a single day. Additionally, implantation of multiple gages within the same animal allows one to measure the relationship between adjacent, or distant, regions of the gastrointestinal tract.

In summary, the fabrication of these subminiature strain gages allows for a wider range of studies utilizing a common array of implantation techniques, instrumentation and data analysis. Among applications across the entire gastrointestinal tract, these gages allow for cross comparison of data collected from A) acute and/or chronic experimental designs; B) multiple (simultaneous) recording sites from within a single animal; and C) a wider range of experimental interventions.

DISCLOSURES

The authors declare that they have no competing financial interests. The suppliers listed in this manuscript are provided for reference only.

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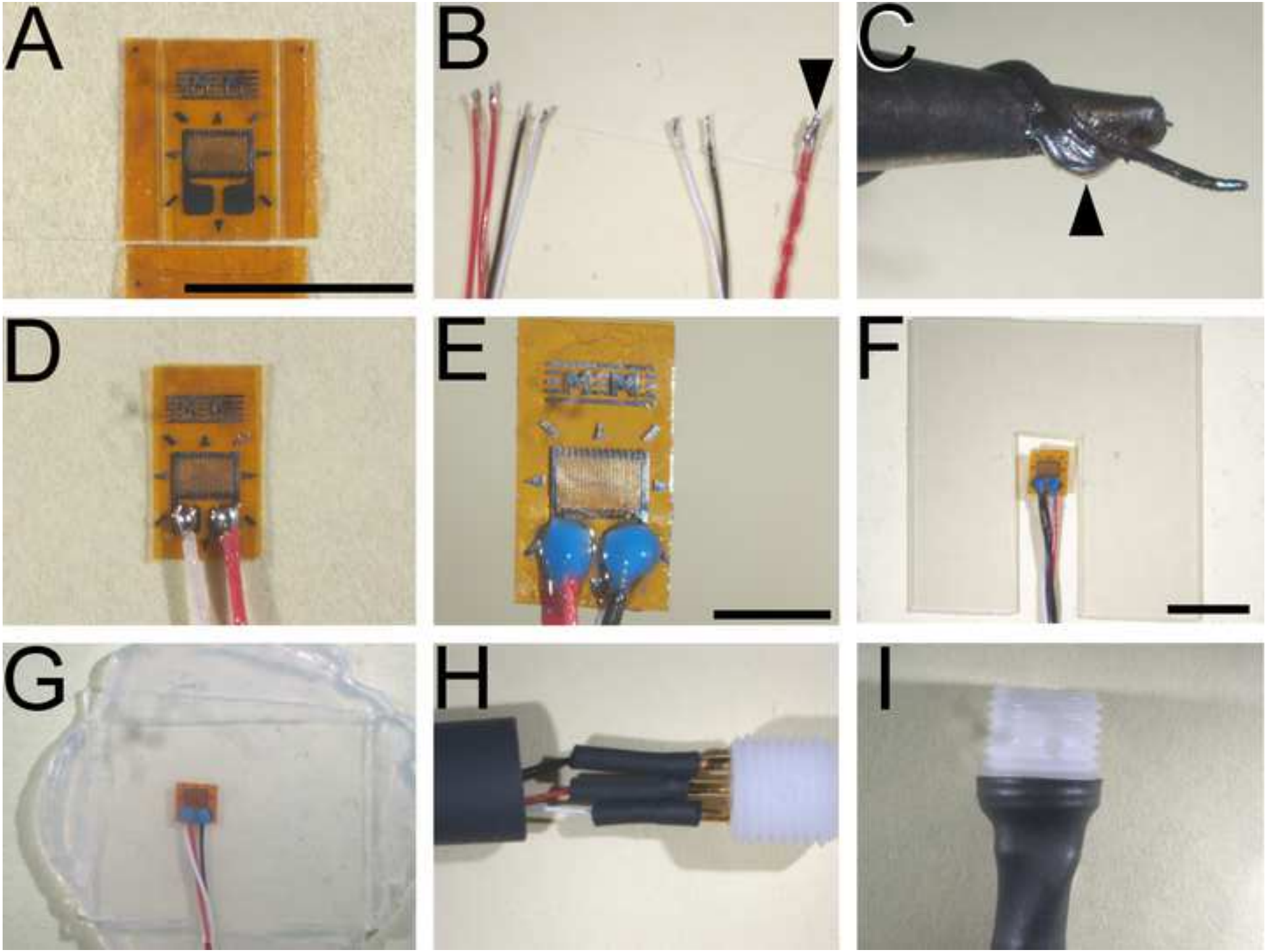

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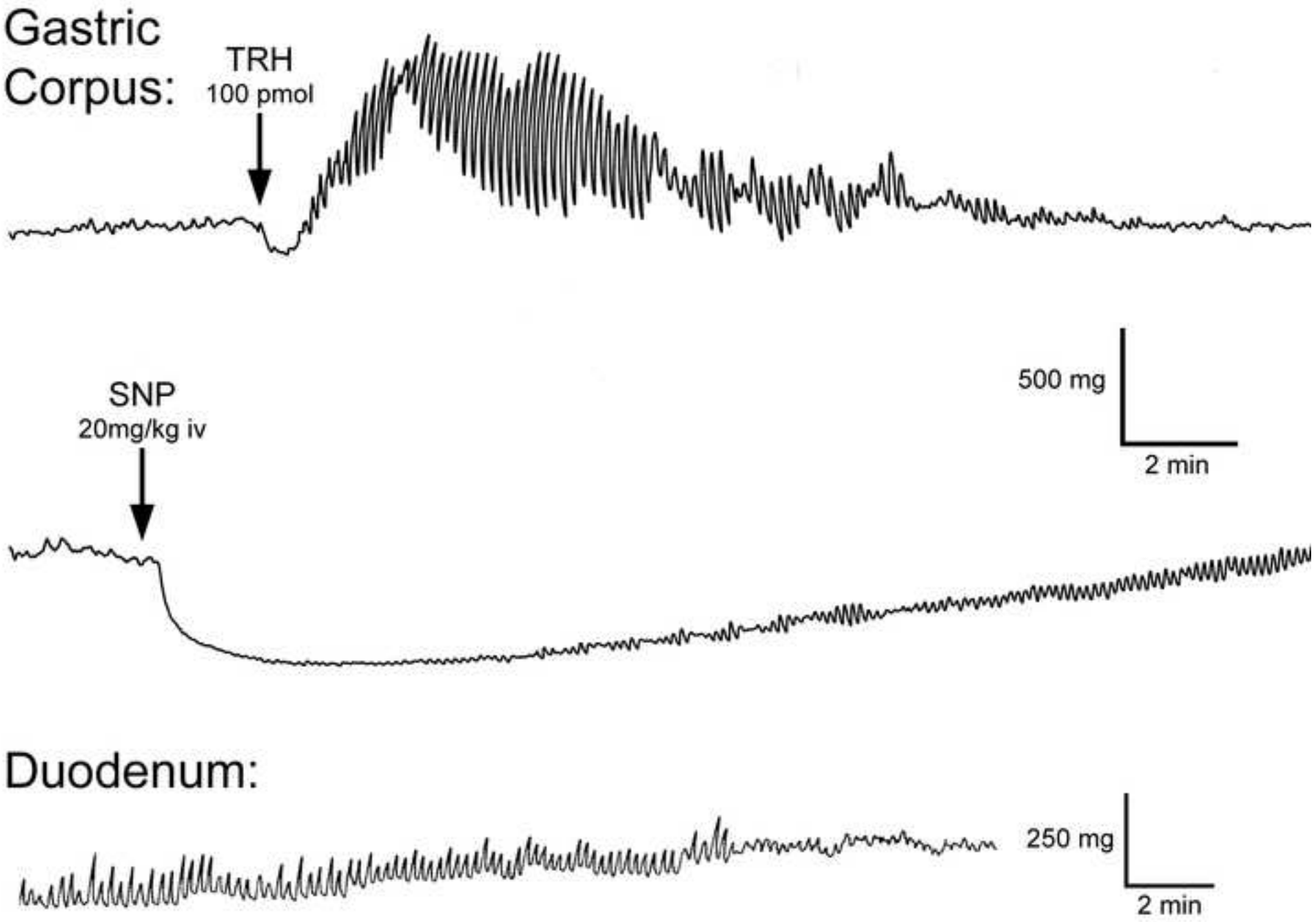

*Excel Spreadsheet- Table of Materials/Equipment

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Component	Company	Part #	Description/purpose	Web URL
Strain gage element	Micro-Measurements (Vishay Product Group)	EA-06-031-350	Linear pattern, foil, stress analysis strain gage (2 required)	www.vishaypg.com/micro-measurements/ or http://www.vishaypg.com/docs/11070/031ce.pdf
epoxy-phenolic adhesive		M-bond 610	General purpose adhesive for bonding strain gage elements	http://www.vishaypg.com/docs/11024/wirecable.pdf
3 conductor insulated wire		336-FTE	Fine gage, flexible general purpose wire	http://www.vishaypg.com/docs/11024/wirecable.pdf
Flux and rosin solvent kit		FAR-2 M-Flux AR kit	Liquid solder flux	http://www.vishaypg.com/docs/11023/soldacce.pdf
Solder		361A-20R-25	Optimized and recommended for strain gage applications	http://www.vishaypg.com/docs/11023/soldacce.pdf
Gold socket connector	PlasticsOne	E363/0	Socket contact for electrode pedestal	http://www.plastics1.com/PCR/Catalog/Item.php?item=407
Electrode pedestal		MS363	Secure platform for wire contacts	http://www.plastics1.com/PCR/Catalog/Item.php?item=499
6-wire cable		363 PLUG W/VINYL SL/6	Pre-fabricated vinyl-coated cable (in customized lengths) with plug adaptor to match electrode pedestal and tinned	

Silicone rubber casting compound	EIS electrical products	Elan Tron E211	Potting medium for gage/wire solder joints	http://www.eis-inc.com
HOTweezers	Meisei Corporation	Model 4B	Wire insulation strippers	http://www.impexron.us
Soldering station	Weller (Apex Tool Group)	WES 51	High quality soldering equipment	http://www.apexhandtools.com/weller/index.cfm Available through http://www.eis-inc.com or http://www.amazon.com
Silicone sheet	Trelleborg Sealing Solutions Northborough-Life Sciences	Pharmelast 20-20	Encapsulating strain gauge elements	10 B Forbes Road Northborough, MA 01532 (800) 634-2000
Amplifier	Experimetria Ltd	AMP-01-SG		http://experimetria.com/Biological_amplifiers.php

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Author(s): Holmes, GM, Swartz EM and McLean, MS

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Department: Neural & Behavioral Sciences

Institution: Penn State University College of Medicine

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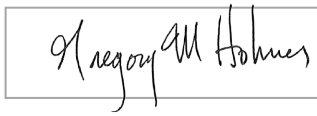
Signature:  Date: 10-21-2013

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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