Dec 19, 2019

Contrast-enhanced magnetic resonance imaging of gastric emptying and motility in rats

Kun-Han Lu[1], Zhongming Liu[1], Jaiyue Cao[1]

[1]Purdue University

1 Works for me dx.doi.org/10.17504/protocols.io.wvxfe7n

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ABSTRACT

Briefly, a gadolinium-based contrast agent was mixed with the animal's meal in order for chyme to appear "bright" in MRI scans, thereby delineating the gastric and intestinal volume. A multi-slice MRI sequence was used to scan the GI volume with high spatial resolution, and a similar sequence with a smaller spatial coverage was used to scan antral contractions with high temporal resolution. Measurements of gastric functions and physiology included the overall change in GI volume, gastric emptying, forestomach volume, corpus volume, antral volume, antral contraction frequency, antral peristaltic wave velocity, antral contraction amplitude, pyloric opening size, intestinal filling and, indirectly, absorption.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Vagus nerve stimulation promotes gastric emptying by increasing pyloric opening measured with magnetic resonance imaging K.-H. Lu J. Cao S. Oleson M. P. Ward R. J. Phillips T. L. Powley Z. Liu. https://doi.org/10.1111/nmo.13380

MATERIALS

NAME	CATALOG #	VENDOR
Sprague-Dawley Rat	RRID:RGD_70508	Envigo
Dietgel	72-06-5022	ClearH20
Gd-DTPA powder	381667	Sigma Aldrich
Carprofen	rimadyldvm	Zoetis
Dexdomitor (dexmedetomidine hydrochloride)	N/A	Zoetis

STEPS MATERIALS

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Animal protocol

1 Thirty rats

Sprague-Dawley Rat
by Envigo
Catalog #: RRID:RGD_70508

(male, 228-330 g) were included in the study according to procedures approved by Purdue Animal Care and Use Committee. Rats were housed individually in ventilated cages with elevated stainless steel wire floors during all time to prevent the animals from accessing their feces. The environment was maintained on a 12:12 hour light-dark cycle (lights on at 6 am and lights off at 6 pm).

Animal and Surgical Steps

2 Each animal was trained to consume a fixed quantity of palatable

Dietgel
by ClearH20
Catalog #: 72-06-5022

The diet training took about 7 days.

In the first 2 days, the animal was supplied with both regular rat chow and ~10g Dietgel (the Dietgel was put in a dish in the cage at 11AM) to accustom itself to the Dietgel.

In the following days, the animal was fasted for 18 hours (5PM to 11AM) and then was fed with the Dietgel only at 11AM. The animal was given 30 minutes to consume 10g Dietgel; then regular meals were supplied to the animal afterwards regardless of whether it finished the Dietgel or not.

After the diet training (~2 to 3 repetitions), each animal was able to naturally consume the Dietgel following overnight food restriction.

On the day for gastric MRI, each animal was given the test meal with a mixture of Dietgel and an MRI contrast agent—Gadolinium (Gd).

Specifically, 10g Dietgel was liquefied through double-boiling in warm water at 45°C and mixed with 22.4 mg of

Gd-DTPA powder
by Sigma Aldrich
Catalog #: 381667

The liquefied Dietgel solution was cooled to room temperature to return it to the semi-solid gel state.

Following an over-night food restriction (18 hours, 5 pm-11 am), rats were able to voluntarily consume the Gd-labeled test meal in 14 ± 5 minutes.

Each animal was then anesthetized with 4% isoflurane mixed with oxygen at a flow rate of 500 mL/min for 5 minutes.

Rats were allocated into 3 groups as follows: (i) an unoperated control group including the rats that did not receive surgery (n = 11); (ii) a sham control group including the rats that only received sham surgery (n = 9) and (iii) a VNS group including the rats that received both surgery and vagus nerve stimulation (VNS) (n = 10).

Rats in the sham and VNS groups underwent the identical neck surgery for implantation of a bipolar cuff electrode around the left cervical vagus nerve. After administering a preoperative bolus of

Carprofen
by Zoetis
Catalog #: rimadyldvm

(10 mg/kg, IP) and performing a toe-pinch to assure adequate anesthesia, a ventral midline cervical incision was made between the mandible and sternum.

Anesthesia was maintained by 2.5%-3.0% isoflurane mixed with oxygen at a flow rate of 1000 mL/min throughout the electrode implant and sham implant surgery.

The subcutaneous tissue of the ventral neck was then bluntly dissected and retracted laterally together with the mandibular salivary glands to reveal the trachea and the left carotid artery.

Upon exposure of the left carotid artery, the left cervical vagus nerve, which sits lateral and runs parallel to the carotid artery above the level of the carotid bifurcation, was identified.

The connective tissues surrounding the left cervical vagus nerve were carefully dissected, so that a 10-15 mm portion of the cervical vagal trunk was isolated from the carotid artery.

A

Custom-designed bipolar cuff electrode
Electrode
MicroProbes custom

with a platinum-iridium wire lead was wrapped and secured on the isolated vagus nerve. The lead was externalized prior to suturing the incision site.

The animal was then placed in prone position on a water-heated MR-compatible cradle.

On the cradle, the animal received a bolus injection of 0.01 mg/kg of

Dexdomitor (dexmedetomidine hydrochloride)
by Zoetis
Catalog #: N/A

solution (0.05 mg/mL, SC.

Five minutes later, the isoflurane dose was reduced to 0.3%-0.5% isoflurane mixed with oxygen at a flow rate of 500 mL/min.

Fifteen minutes after the initial bolus, a continuous subcutaneous infusion of Dexdomitor was administered (0.03 mg/kg/h, SC).

A

Model 1030 MR Compatible Monitoring and Gating System
Monitoring System
Small Animal Instruments Inc -

was used to monitor respiration, cardiac pulsation, and body temperature to ensure a stable physiological state throughout the experiment.

The 2 leads of the vagal electrodes were connected to a pair of twisted wires that ran from the MRI bore to the console room, and the wires were further connected to a

Constant-current Stimulator
Stimulator
A-M Systems Model 2200

Upon the start of the first MRI acquisition, electrical pulses (monophasic pulses with alternating polarity, inter-pulse duration = 50 ms; pulse amplitude = 0.6 mA; pulse width = 0.36 ms; frequency = 10 Hz; 20 seconds on and 40 seconds off) were delivered to the cervical vagus throughout the 4-hour experiment.

Gastric MRI

3 The animals were scanned in a 7-tesla horizontal-bore small animal MRI system

BioSpec 70/30
7T MRI
Bruker -

equipped with a gradient insert (maximum gradient: 200 mT/m; maximum slew rate: 640 T/m/s) and a volume transmit/receive 1H RF coil (86 mm inner-diameter). As in our earlier study, after the long axis of the stomach was localized with the initial MRI scans, each animal was imaged with a series of alternating volumetric scans and fast scans; the former was for quantifying gastric volume with higher spatial resolution and larger spatial coverage, whereas the latter was for assessing antral motility with higher temporal resolution and more targeted spatial coverage. The volumetric scans were acquired using a 2-dimensional Fast Low Angle Shot gradient echo (FLASH) sequence with repetition time (TR) = 124.131 ms, echo time (TE) = 1.364 ms, flip angle (FA) = 90°, 30 oblique slices, slice thickness = 1 mm, field of view (FOV) = 60 × 60 mm^2, in-plane resolution = 0.23 × 0.23 mm^2, and 4 averages. The fast scans were acquired using a 2-dimensional FLASH sequence with TR/TE = 11.784/1.09 ms, FA = 25°, 4 oblique slices, slice thickness = 1.5 mm, FOV = 60 × 60 mm^2, in-plane resolution 0.47 × 0.47 mm^2, no averaging, and 150 repetitions. The 4 fast scan slices were positioned and adjusted to cover the antrum, pylorus and duodenum, based on the immediately preceding volumetric images to account for the stomach displacement during gastric emptying.To minimize motion artifacts, both volumetric and fast scans were respiratory-gated, such that images were acquired during end-expiratory periods, while the chest volume stayed roughly unchanged. With the respiratory gating, the volumetric scan took about 4 minutes; the fast scan took ~2 seconds per repetition and lasted ~6 minutes for 150 repetitions. The volumetric and fast scans were repeated in an interleaved manner for a total of 4 hours.

Assessment of GI volume, compartmental volume, and emptying rate

4 The GI volume was assessed globally and compartmentally, which included the gastric volume and intestinal volume. The intestinal volume comprised the duodenal, jejunal, and ileal volumes. The gastric volume was further partitioned into forestomach, corpus and antral volumes. The volumes were sampled approximately every 15 minutes for 4 hours. Specifically, the contrast-enhanced lumenal volumes of the GI tract at different times were segmented, partitioned, and quantified separately from the volumetric scans by using an image processing pipeline. Note that some voxels in large veins with much shortened T1 and those in the renal medulla because of systemic Gd absorption might be mistakenly included in the above segmentation. Such spurious voxels were manually identified and excluded from the analysis. In addition, the heterogeneous image intensity (e.g. feces with unenhanced and/or partly enhanced image intensity) in the colon raised difficulty in proper quantification of the colonic volume; hence, voxels in the colon were removed as well. The processing time for a 4-hour volumetric dataset was about 1.5 hours. The volume of each compartment measured at intervals was further normalized as a percent of its initial volume at time 0. This normalization step allowed us to observe the relative volume change over time for each animal, while accounting for the varying amount of the meal intake and the preparation time for different animals. The time series of gastric volumes was resampled at 15-min intervals for every animal and then averaged across animals to characterize gastric emptying at the group level. Note that the VNS began at the start of the first volumetric scan (t = 0). As the scan took about 4 minutes to acquire, a 4-minute delay was added to all time series.

Assessment of gastric motility

5 The frequency, amplitude, and velocity of the peristaltic wave in the gastric antrum were quantified from the fast scans by using a custom-built Matlab image processing pipeline. Briefly, the antrum was first delineated from a stack of 4 slices, and the proximal-to-distal antral axis was determined. The cross-sectional areas (CSA) perpendicular to the proximal-to-distal antral axis were then calculated by summing the number of antral voxels within each cross-sectional plane. By iteratively doing so for each volume, we obtained a time series that represented the CSA change of the antrum at different locations distant to the pylorus. In the CSA time series, the maxima of the time series indicated antral distension and the minima antral contraction (ie, the lumen was largely occluded by the depth of the constriction of the antral wall). The antral contraction frequency, occlusion amplitude, and velocity were computed from the time series. In this study, the antral motility indices were obtained from the middle antrum, which was 4.7 mm distant from the pylorus. This process was repeated for each volume. A time series that characterized the antral motility was obtained and resampled at 15-min intervals for every animal and then averaged across animals. As VNS began at the start of the first volumetric scan (t = 0) and the volumetric scan and the fast scan took about 4 and 6 minutes, respectively, a 10-minute delay was added to all time series.

Measurement of the size of the pyloric sphincter lumen

6 To measure the size of the pyloric sphincter, we manually determined a cross-sectional plane that was perpendicular to the outflow direction of the terminal antrum on the segmented GI tract from the volumetric scans. The CSA of the pyloric sphincter was calculated by counting the number of lumenal voxels in the determined plane. This process was repeated for each volume, and a time series that characterized the pyloric opening was obtained for each animal. The time series was resampled at 15-minute intervals for every animal and then averaged across animals for analysis at the group level. A 4-minute delay was added to all time series for the same reason mentioned in the gastric emptying analysis.

Statistical analysis

7 Unless otherwise stated, all data are reported as mean±standard error of mean (SEM). A probability (P-value) <.05 was considered significant to reject the null hypothesis. To evaluate the significance of the difference in the gastric emptying profile among the 3 conditions, the emptying curve from each subject was modeled by a Weibull distribution expressed as below, for which the 2 parameters (t_{const}, β) were estimated by the least-squares method,$V(t)(\%) = 100\exp(-\beta(t/t_{const}))$ where V(t) is the remaining volume at experiment time t (min), β is the shape parameter of the curve, and t_{const} is the emptying time constant (minute). The fitting was done by using the fit function in Matlab. Only the estimated parameters with goodness of fit (R^2) metrics greater than 0.85 were subject to the subsequent statistical analysis. One-way ANOVA was performed to assess the significance of the differences between the fitted parameters for the unoperated control, the sham control, and the VNS conditions, followed by Fisher's least significant difference (LSD) post hoc tests. To further assess the difference of the remaining volume in each compartment, one-way ANOVA with LSD test was conducted on individual time points between the 3 conditions. One-way ANOVA with LSD test was also applied to determine whether there were statistically significant differences in antral motility indices and the degree of the pyloric opening between the 3 conditions.