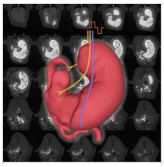

May 04, 2021

Effects of Vagus Nerve Stimulation/Gastric Electrical Stimulation on Gastric Emptying and Motility Assessed with Magnetic Resonance Imaging

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

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ABSTRACT

This protocol describes the methods used to evaluate the effects of vagus nerve stimulation (VNS) and gastric electrical stimulation (GES) settings on gastric emptying and motility in rats. 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 gastrointestinal (GI) functions and physiology with high spatial and temporal resolution.

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PROTOCOL CITATION

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KEYWORDS

Magnetic Resonance Imaging, Vagus Nerve Stimulation, Gastric Electrical Stimulation, Gastric Emptying, Gastric Motility, Rat

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Acute effects of vagus nerve stimulation settings on gastric motility assessed with MRI

1 **Study title**

Acute effects of vagus nerve stimulation settings on gastric motility assessed with MRI

Description

This protocol describes the methods used to evaluate the effects of VNS settings on gastric motility in rats. Briefly, a

Citation: Kun-Han Lu, Zhongming Liu, Jiayue Cao (05/04/2021). Effects of Vagus Nerve Stimulation/Gastric Electrical Stimulation on Gastric Emptying and Motility Assessed with Magnetic Resonance Imaging. https://dx.doi.org/10.17504/protocols.io.bawfifbn

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 antral contractions with high spatial and temporal resolution. The configuration of VNS was varied in terms of pulse parameters (amplitude, width, and frequency) and polarity (proximal or distal coil of a bipolar electrode connected as the cathode, which could be biased for afferent or efferent VNS).

1.1 Animal protocol

Twelve rats
Sprague-Dawley
Rat **Envigo Catalog #RRID:RGD_70508**
(Male), ranging from 266 to 338g body weight were used in this study. Meal preparation, animal preparation, surgical steps, imaging sequence, data analysis were performed using our previously published protocol. Note that no Carprofen was administered to the animals in this study.

1.1.1 Thirty rats
Sprague-Dawley
Rat **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).

1.1.2 Each animal was trained to consume a fixed quantity of palatable
Dietgel **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 **Sigma Aldrich Catalog #381667**

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

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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 **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) **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

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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.

1.1.3 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.

The GI volume was assessed globally and compartmentally, which included the gastric volume and intestinal volume.

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1.1.4 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.

1.1.5 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.

1.1.6 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.

1.1.7 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.

1.2 Experiment Design

After obtaining about 5 minutes of stable, baseline dynamic MRI images, cervical VNS was delivered simultaneously with MRI acquisition. The 12 rats were allocated into 2 groups as follows: (i) a group of

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rats (N=7) that received afferent VNS (i.e. cathode placed proximally to the anode) and (ii) a group of rats (N=5) that received efferent VNS (i.e. cathode placed distally to the anode). In addition to evaluating the effect of afferent versus efferent VNS on gastric motility, we further applied bi-directional VNS (i.e. combined afferent and efferent VNS) to the rats in the afferent VNS group, immediately after which afferent VNS was performed. The bi-directional VNS was achieved by delivering monophasic pulses of current in alternating directions between the two electrodes.

For each VNS group, the stimulus parameters were varied in terms of pulse amplitude (PA: 0.13, 0.25, 0.5, 1mA), width (PW: 0.13, 0.25, 0.5ms), and frequency (PF: 5, 10Hz). The frequency of afferent or efferent VNS was defined as the number of electrical pulses delivered per second. The frequency of bidirectional VNS was defined as the number of paired cathodal and anodal pulses delivered per second. Low, medium and high values were selected for each parameter settings, all of which are frequently used in clinical and preclinical settings. Each stimulation setting comprised of a duty cycle of 1-minute ON and 1-minute OFF, and different VNS settings were performed in a randomized order to eliminate any causal effect of one setting on the other. No stimulus was delivered during the OFF period. As a result, every rat in each group underwent VNS with 24 different sets of parameters.

Effects of nodose ganglion blockade on gastric motility during cervical vagus nerve stimulation measured with MRI

2 Study title

Effects of nodose ganglion blockade on gastric motility during cervical vagus nerve stimulation measured with MRI

Description

This protocol describes the methods used to evaluate the effects of cervical VNS on gastric motility in rats. Gastric MRI was performed during cervical VNS with and without afferent blockade at the nodose ganglion.

2.1 Animal protocol

Eight rats

Sprague-Dawley

Rat **Envigo Catalog #RRID:RGD_70508**

(Male), ranging from 283 to 376g body weight were used in this study. Meal preparation, animal preparation, surgical steps, imaging sequence, data analysis were performed using our previously published protocol. Note that no Carprofen was administered to the animals in this study.

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

PREVIEW RUN

2.1.1

Thirty rats

Sprague-Dawley

Rat **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).

2.1.2

Each animal was trained to consume a fixed quantity of palatable

Dietgel **ClearH20 Catalog #72-06-5022**

The diet training took about 7 days.

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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 **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 **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

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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) **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.

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

BioSpec 70/30
7T MRI
Bruker -

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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.

2.1.4 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.

2.1.5 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.

2.1.6 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.

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2.1.7 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.

2.2 Experiment Design

The 8 rats were allocated into 2 groups as follows: (i) a group of rats (N=4) that did not receive injection 1.5uL of 2% Lidocaine

Lidocaine

2% **VetOne Catalog #V1 510212**

into the left nodose ganglion and (ii) a group of rats (N=4) that received injection 1.5uL of 2% Lidocaine into the left nodose ganglion. After obtaining about 4 minutes of stable, baseline dynamic MRI images, cervical VNS (0.3mA, 0.2ms, 10Hz, 20s ON & 40s OFF) was delivered for 5 minutes simultaneously with MRI acquisition. The cathode was set cranial to the cuff to favor activation of afferent signaling to the brain. MRI images were collected for an additional 20 minutes after the offset of VNS.

Acute effects of gastric electrical stimulation settings on gastric motility assessed with MRI

3 Study title

Acute effects of gastric electrical stimulation settings on gastric motility assessed with MRI

Description

This protocol describes the methods used to evaluate the effects of GES settings on gastric motility in rats. 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 antral contractions with high spatial and temporal resolution. The configuration of VNS was varied in terms of pulse parameters (amplitude, width, and frequency).

3.1 Animal protocol

Four rats

Sprague-Dawley

Rat **Envigo Catalog #RRID:RGD_70508**

(Male), ranging from 272 to 306g body weight were used in this study. All animals underwent an abdominal surgery for implantation of a patch electrode on the forestomach. Each animal was briefly anesthetized with 5% Isoflurane for 5 min, followed by 2% isoflurane to maintain a surgical plane of anesthesia. Following a toe-pinch test, a ~3 cm incision was made starting at 1 cm caudal to the xiphoid and moving 3 cm caudally. After separating and retracting the skin and muscle layers, the ventral stomach was exposed such that the intersection of the greater curvature and the limiting ridge could be identified. A pair of customized electrodes

Citation: Kun-Han Lu, Zhongming Liu, Jiayue Cao (05/04/2021). Effects of Vagus Nerve Stimulation/Gastric Electrical Stimulation on Gastric Emptying and Motility Assessed with Magnetic Resonance Imaging. https://dx.doi.org/10.17504/protocols.io.bawfifbn

Customized bipolar patch electrode
Microprobes N/A

which consisted of Pt/Ir foils adhered to a thin perylene substrate (a rectangular shape of 4 mm-by-2mm size and 0.015 mm thickness), was directly sutured onto the forestomach wall. This patch electrode was placed along the greater curvature at about 4 mm proximal to the limiting ridge. Next, the muscle and skin layers at the incision site were closed with sutures. The leads of the implanted electrodes were tunneled subcutaneously to the back. The animals were allowed at least 1 week for post-surgical recovery. To minimize pain and inflammatory responses after the surgery, all animals were given

Baytril **Bio-Serv Catalog #F06801**

(one tablet per day, 2 mg/Tablet) two days before the surgery and

Buprenex (buprenorphine hydrochloride) **Contributed by users**

(0.01 mg/kg, subcutaneous) immediately before the surgery. Meal preparation, animal preparation, imaging sequence, data analysis were performed using our previously published protocol. Note that no Carprofen was administered to the animals in this study.

Contrast-enhanced magnetic resonance imaging of gastric emptying and motility in rats
by Deborah Jaffey
PREVIEW RUN

3.1.1 Thirty rats

Sprague-Dawley

Rat **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).

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

Dietgel **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).

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

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(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.

3.1.3

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

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repetitions. The volumetric and fast scans were repeated in an interleaved manner for a total of 4 hours.

3.1.4 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.

3.1.5 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.

3.1.6 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.

3.1.7 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.

3.2 Experiment Design

Citation: Kun-Han Lu, Zhongming Liu, Jiayue Cao (05/04/2021). Effects of Vagus Nerve Stimulation/Gastric Electrical Stimulation on Gastric Emptying and Motility Assessed with Magnetic Resonance Imaging. https://dx.doi.org/10.17504/protocols.io.bawfifbn

After obtaining about 5 minutes of stable, baseline dynamic MRI images, gastric electrical stimulation was delivered simultaneously with MRI acquisition. Monophasic pulses of current was delivered in alternating directions between the two electrodes.The stimulus parameters were varied in terms of pulse amplitude (PA: 0, 0.2, 0.4, 0.6, 0.8, 1mA), width (PW: 0.1, 0.2, 0.4ms), and frequency (PF: 2, 5, 10, 20Hz). The frequency of GES was defined as the number of paired cathodal and anodal pulses delivered per second. Low, medium and high values were selected for each parameter settings, all of which are frequently used in clinical and preclinical settings. Each stimulation setting comprised of a duty cycle of 20s ON and 40s OFF, and different GES settings were performed in a randomized order to eliminate any causal effect of one setting on the other. No stimulus was delivered during the OFF period. As a result, every rat underwent GES with 72 different sets of parameters.

Assessment of gastric emptying and motility with MRI under gastric electrical stimulation

4 Study title

Assessment of gastric emptying and motility with MRI under gastric electrical stimulation

Description

This protocol describes the methods used to evaluate the effects of GES on gastric emptying and motility in rats.

4.1 Animal protocol

Eleven rats

☒ Sprague-Dawley

Rat **Envigo Catalog #RRID:RGD_70508**

(Male), ranging from 272 to 407g body weight were used in this study. All animals underwent an abdominal surgery for implantation of a patch electrode on the forestomach. Each animal was briefly anesthetized with 5% Isoflurane for 5 min, followed by 2% isoflurane to maintain a surgical plane of anesthesia. Following a toe-pinch test, a ~3 cm incision was made starting at 1 cm caudal to the xiphoid and moving 3 cm caudally. After separating and retracting the skin and muscle layers, the ventral stomach was exposed such that the intersection of the greater curvature and the limiting ridge could be identified. A pair of customized electrodes

Customized bipolar patch electrode	
Microprobes	**N/A**

which consisted of Pt/Ir foils adhered to a thin perylene substrate (a rectangular shape of 4 mm-by-2mm size and 0.015 mm thickness), was directly sutured onto the forestomach wall. This patch electrode was placed along the greater curvature at about 4 mm proximal to the limiting ridge. Next, the muscle and skin layers at the incision site were closed with sutures. The leads of the implanted electrodes were tunneled subcutaneously to the back. The animals were allowed at least 1 week for post-surgical recovery. To minimize pain and inflammatory responses after the surgery, all animals were given

☒ Baytril **Bio-**

Serv Catalog #F06801

(one tablet per day, 2 mg/Tablet) two days before the surgery and

☒ Buprenex (buprenorphine hydrochloride) **Contributed by users**

(0.01 mg/kg, subcutaneous) immediately before the surgery. Meal preparation, animal preparation, imaging sequence, data analysis were performed using our previously published protocol. Note that no Carprofen was administered to the animals in this study.

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4.1.1 Thirty rats
Sprague-Dawley
Rat **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).

4.1.2 Each animal was trained to consume a fixed quantity of palatable
Dietgel **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 **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 **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.

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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) **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

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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.

4.1.3 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.

4.1.4 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-

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minute delay was added to all time series.

4.1.5 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.

4.1.6 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.

4.1.7 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.

4.2 Experiment Design

The 11 rats were allocated into 3 groups as follows: (i) a group of rats (N=5) that did not receive GES, (ii) a group of rats (N=2) that received GES with stimulus setting being pulse amplitude=0.3mA, pulse width=0.2ms, pulse frequency=10Hz, and (iii) a group of rats (N=4) that received GES with stimulus setting being pulse amplitude=0.6mA, pulse width=0.2ms, pulse frequency=5Hz. Monophasic pulses of current was delivered in alternating directions between the two electrodes. Gastric electrical stimulation was delivered simultaneously with MRI acquisition. Volumetric and motility MRI imaging were performed in an interleaved manner continuously for 4 hours.

Effects of vagal afferent blockade on gastric motility during cervical vagus nerve stimulation measured with MRI

5 Study title

Effects of vagal afferent blockade on gastric motility during cervical vagus nerve stimulation measured with MRI

Description

This protocol describes the methods used to evaluate the effects of cervical VNS on gastric motility in rats. The aim is to selectively activate or block afferent pathway to disentangle their differential effects on gastric responses.

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5.1 Animal protocol

Fourteen rats
Sprague-Dawley
Rat **Envigo Catalog #RRID:RGD_70508**
(Male), ranging from 242 to 327g body weight were used in this study. Meal preparation, animal preparation, surgical steps, imaging sequence, data analysis were performed using our previously published protocol. In addition, a cuff catheter was placed cranial to the stimulation cuff electrode.Note that no Carprofen was administered to the animals in this study.

Contrast-enhanced magnetic resonance imaging of gastric emptying and motility in rats
by Deborah Jaffey
PREVIEW RUN

5.1.1

Thirty rats
Sprague-Dawley
Rat **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).

5.1.2

Each animal was trained to consume a fixed quantity of palatable
Dietgel **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 **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.

Citation: Kun-Han Lu, Zhongming Liu, Jiayue Cao (05/04/2021). Effects of Vagus Nerve Stimulation/Gastric Electrical Stimulation on Gastric Emptying and Motility Assessed with Magnetic Resonance Imaging. https://dx.doi.org/10.17504/protocols.io.bawfifbn

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 **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) **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 -

Citation: Kun-Han Lu, Zhongming Liu, Jiayue Cao (05/04/2021). Effects of Vagus Nerve Stimulation/Gastric Electrical Stimulation on Gastric Emptying and Motility Assessed with Magnetic Resonance Imaging. https://dx.doi.org/10.17504/protocols.io.bawfifbn

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.

5.1.3 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.

5.1.4 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

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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.

5.1.5 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.

5.1.6 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.

5.1.7 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.

5.2 Experiment Design

The 14 rats were allocated into 2 groups as follows: (i) a group of rats (N=7) that did not receive infusion of 2% Lidocaine

Lidocaine

2% **VetOne Catalog #V1 510212**

onto the left cervical vagus and (ii) a group of rats (N=7) that received infusion of 2% Lidocaine onto the left cervical vagus. The infusion protocol consists an initial fast infusion of 2% Lidocaine at 0.3mL/h for 6 minutes, followed by continuous infusion at a rate of 0.03ml/h. As soon as the initial fast infusion was done, baseline dynamic MRI images were acquired for 15 minutes. Then, cervical VNS (0.25mA, 0.5ms, 5Hz, 20s ON & 40s OFF) was delivered for 15 minutes simultaneously with MRI acquisition. The cathode was set cranial to the cuff to favor activation of afferent signaling to the

Citation: Kun-Han Lu, Zhongming Liu, Jiayue Cao (05/04/2021). Effects of Vagus Nerve Stimulation/Gastric Electrical Stimulation on Gastric Emptying and Motility Assessed with Magnetic Resonance Imaging. https://dx.doi.org/10.17504/protocols.io.bawfifbn

brain. MRI images were collected for an additional 50 minutes after the offset of VNS.

Effects of vagal efferent blockade on gastric motility and emptying during cervical vagus nerve stimulation measured with MRI

6 Study title

Effects of vagal efferent blockade on gastric motility and emptying during cervical vagus nerve stimulation measured with MRI

Description

This protocol describes the methods used to evaluate the effects of cervical VNS on gastric emptying and motility in rats. The aim is to selectively activate afferent pathway (with and without blockade of efferent pathway) to disentangle their differential effects on gastric responses.

6.1 Animal protocol

Nine rats

Sprague-Dawley

Rat **Envigo Catalog #RRID:RGD_70508**

(Male), ranging from 252 to 366g body weight were used in this study. Meal preparation, animal preparation, surgical steps, imaging sequence, data analysis were performed using our previously published protocol. In addition, a cuff catheter was placed caudal to the stimulation cuff electrode. Note that no Carprofen was administered to the animals in this study.

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

PREVIEW RUN

6.1.1 Thirty rats

Sprague-Dawley

Rat **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).

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

Dietgel **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—

Citation: Kun-Han Lu, Zhongming Liu, Jiayue Cao (05/04/2021). Effects of Vagus Nerve Stimulation/Gastric Electrical Stimulation on Gastric Emptying and Motility Assessed with Magnetic Resonance Imaging. https://dx.doi.org/10.17504/protocols.io.bawfifbn

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 **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 **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) **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.

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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.

6.1.3 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

Citation: Kun-Han Lu, Zhongming Liu, Jiayue Cao (05/04/2021). Effects of Vagus Nerve Stimulation/Gastric Electrical Stimulation on Gastric Emptying and Motility Assessed with Magnetic Resonance Imaging. https://dx.doi.org/10.17504/protocols.io.bawfifbn

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.

6.1.4 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.

6.1.5 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.

6.1.6 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.

6.1.7 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.

Citation: Kun-Han Lu, Zhongming Liu, Jiayue Cao (05/04/2021). Effects of Vagus Nerve Stimulation/Gastric Electrical Stimulation on Gastric Emptying and Motility Assessed with Magnetic Resonance Imaging. https://dx.doi.org/10.17504/protocols.io.bawfifbn

6.2 Experiment Design

The 9 rats were allocated into 2 groups as follows: (i) a group of rats (N=5) that did not receive infusion of 2% Lidocaine

Lidocaine

2% **VetOne Catalog #V1 510212**

onto the left cervical vagus and (ii) a group of rats (N=4) that received infusion of 2% Lidocaine onto the left cervical vagus. The infusion protocol consists an initial fast infusion of 2% Lidocaine at 0.3mL/h for 6 minutes, followed by continuous infusion at a rate of 0.03ml/h. As soon as the initial fast infusion was done, volumetric and motility MRI imaging were performed in an interleaved manner continuously for 4 hours. Cervical VNS (0.25mA, 0.5ms, 5Hz, 20s ON & 40s OFF) was continuously with MRI acquisition. The cathode was set cranial to the cuff to favor activation of afferent signaling to the brain.

In vivo mapping of gastric electrical activation with manganese-enhanced magnetic resonance imagingSPARC - OT2OD023847 - SA2.1b - Y3Q4

7 Study title

In vivo mapping of gastric electrical activation with manganese-enhanced magnetic resonance imaging

Description

This protocol describes the use of manganese-enhanced MRI to map gastric electrical activation following meal consumption. The main classes of ion channels expressed in GI smooth muscle mirror those in vascular smooth muscle cells (SMCs) and include similar K^+ channel families that set the resting membrane potentials and buffer contraction and the voltage-gated calcium channels and cationic TRPC channels that mediate SMC contraction. To assess intracellular calcium, one possible technique is to use manganese ion that has physical properties similar to calcium that can be handled similarly in many biological systems. Manganese ion is also an excellent T_1 contrast agent.

Imaging manganese with MRI showed elevated calcium activity confined to the antrum and corpus.

7.1 Animal protocol

One rat

Sprague-Dawley

Rat **Envigo Catalog #RRID:RGD_70508**

(Male, 342g) was used in the study according to procedures approved by the 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).

The animal was trained to consume a fixed quantity of palatable Dietgel.

Dietgel **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 afterward regardless of whether it finished the Dietgel or not. After the diet training (~2 to 3 repetitions), the animal was able to naturally consume the Dietgel following overnight food restriction. On the day for manganese-enhanced magnetic resonance imaging (MEMRI), the animal was given 5g of the test meal.

Animal preparation and imaging protocol were performed using our previously published protocol.

Citation: Kun-Han Lu, Zhongming Liu, Jiayue Cao (05/04/2021). Effects of Vagus Nerve Stimulation/Gastric Electrical Stimulation on Gastric Emptying and Motility Assessed with Magnetic Resonance Imaging. https://dx.doi.org/10.17504/protocols.io.bawfifbn

7.1.1 Thirty rats
Sprague-Dawley
Rat **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).

7.1.2 Each animal was trained to consume a fixed quantity of palatable
Dietgel **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 **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 **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.

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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) **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

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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.

7.1.3 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.

7.1.4 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-

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minute delay was added to all time series.

7.1.5 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.

7.1.6 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.

7.1.7 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.

7.2 Experiment Design

Pre-contrast volumetric gastric MRI images were collected prior to the onset of $MnCl_2$ infusion. Then, 100mM $MnCl_2$

☒ Manganese(II) chloride tetrahydrate **Sigma Aldrich Catalog #M3634**

was systemically infused through the tail vein at a rate of 0.5ml/h for 20 minutes. Dynamic contrast-enhanced gastric MRI images were acquired continuously for 80 minutes.

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