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# Electrical colonic stimulation reduces mean transit time in a porcine model

THESE

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***Electrical colonic stimulation reduces mean transit time in a porcine model***

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## Rapport de synthèse

### Introduction

La stimulation électrique représente une nouvelle modalité thérapeutique de divers troubles digestifs. Dans la constipation par exemple, le péristaltisme colique peut être activé par un système électrique alimenté par une batterie. La présente étude a pour but de démontrer l'impact d'une stimulation électrique directe du côlon sur le temps de transit moyen, en utilisant un modèle expérimental chronique porcin. L'effet de la stimulation et du matériel implanté dans la paroi colique est également évalué.

### Matériel et méthode

Trois paires d'électrodes ont été implantées dans la paroi cæcale de douze porcs anesthésiés. Avant implantation, un temps de transit colique de référence a été déterminé chez chaque animal par utilisation de marqueurs radio-opaques. Cette évaluation a été répétée quatre semaines après implantation, sous stimulation factice, et cinq semaines après implantation, sous stimulation électrique. Des trains séquentiels et aboraux de stimulation (10 V ; 120 Hz ; 1 ms) ont été appliqués quotidiennement durant six jours, en utilisant un stimulateur externe fonctionnant sur batteries. Pour chaque série de marqueurs, une valeur moyenne a été calculée à partir du temps de transit individuel des porcs. Un examen microscopique du cæcum a été systématiquement entrepris après sacrifice des animaux.

### Résultats

Une réduction du temps de transit moyen a été observée après stimulation électrique (19h ± 13 ; moyenne ± DS), comparativement au temps de référence (34h ± 7 ; $p=0.045$) et au temps de transit après stimulation factice (36h ± 9 ; $p=0.035$). L'examen histologique a montré la présence d'une inflammation chronique minime, autour des électrodes.

### Conclusion

Le temps de transit colique porcin peut être réduit, en conditions expérimentales chroniques, par une stimulation électrique directe et séquentielle de l'intestin. Des lésions tissulaires limitées ont été occasionnées par la stimulation ou le matériel implanté. La stimulation électrique colique représente certainement une approche prometteuse du traitement de certains troubles spécifiques du côlon, avant tout fonctionnels.

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# Electrical colonic stimulation reduces mean transit time in a porcine model

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***Abstract*** *Electrical stimulation is a new way to treat digestive disorders such as constipation. Colonic propulsive activity can be triggered by battery operated devices. This study aimed to demonstrate the effect of direct electrical colonic stimulation on mean transit time in a chronic porcine model. The impact of stimulation and implanted material on the colonic wall was also assessed. Three pairs of electrodes were implanted into the caecal wall of 12 anaesthetized pigs. Reference colonic transit time was determined by radiopaque markers for each pig before implantation. It was repeated 4 weeks after implantation with sham stimulation and 5 weeks after implantation with electrical stimulation. Aboral sequential trains of 1-ms pulse width (10 V; 120 Hz) were applied twice daily for 6 days, using an external battery operated stimulator. For each course of markers, a mean value was computed from transit times obtained from individual pig. Microscopic examination of the caecum was routinely performed after animal sacrifice. A reduction of mean transit time was observed after electrical stimulation (19 ± 13 h; mean ± SD) when compared to reference (34 ± 7 h; P = 0.045) and mean transit time after sham stimulation (36 ± 9 h; P = 0.035). Histological examination revealed minimal chronic inflammation around the electrodes. Colonic transit time measured in a chronic porcine model is reduced by direct sequential electrical stimulation. Minimal tissue lesion is elicited by stimulation or implanted material. Electrical colonic stimulation could be a promising approach to treat specific disorders of the large bowel.*

***Keywords*** *chronic experimentation, colorectal motility, constipation, electrical stimulation.*

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## INTRODUCTION

Electrical stimulation of the gastrointestinal tract has been studied for many years. Different techniques have been tested on various digestive segments to activate or inhibit the motor function.[1] While gastric stimulation has become a treatment option for gastroparesis, direct stimulation of other parts of the digestive tract still remains experimental.[2]

Electrical colonic stimulation has been described in experimental models using various parameters (Table 1). Bruninga *et al.* performed acute experiments on a cat model with spinal cord injury. Colonic transit was improved, as measured by radiopaque markers and manometry.[3] Amaris *et al.* described an accelerated progression of the content in transected canine descending colon.[4] Sanmiguel *et al.* performed chronic experiments, 2 weeks after surgery, in dogs with delayed colonic transit.[5] They showed that stimulation induced strong sequential colonic contractions and significantly accelerated content movement, confirmed by radiopaque markers and manometry. Sevcencu *et al.* observed colonic contractions by impedance planimetry and manometry in a porcine model.[6] Only Shafik *et al.* applied direct stimulation on human colon.[7] Mucosal electrodes were placed endoscopically in nine constipated patients with colonic inertia. Pacing was performed via an inguinal

**Table 1** Studies reporting colonic electrical stimulation

| | | | Electrodes | | Stimulation parameters | | | |
|---|---|---|---|---|---|---|---|---|
| | Experimental model/nb. | Study design | Colonic localisation | *n* | Pulse width (ms) | Frequency (Hz) | Amplitude | Sequential stimulation |
| Bruninga *et al.*[3] | Cat/5 | Acute | Transverse and descending | 3 pairs | 0.1–1 | 10–40 | 0–50 mA | No |
| Amaris *et al.*[4] | Dog/6 | Acute | Descending | 4 pairs | 10 | 50 | 20 V | Yes |
| Sevcencu *et al.*[6] | Pig/8 | Acute | Descending | 3 electrodes* | 0.03, 0.3, 3 | 10 | 9, 12, 15, 30 mA | Yes |
| Sanmiguel *et al.*[5] | Dog/9 | Chronic | Distal colon | 4 pairs | 10 | 50 | 7.8–9.9 mA 8–10 V | Yes |
| Aellen *et al.*[8] | Pig/8 | Acute | Caecum | 3 pairs | 0, 2–1 | 5–120 | 7–15 mA 1–10 V | Yes |

*Each electrode exhibits three deinsulated sites.

subcutaneous stimulator. An increase in defecation frequency was observed in two thirds of the patients at 6–14 months.

In acute experiments on the pig caecum, our group has demonstrated the feasibility of a battery operated stimulator to generate contractions and has determined optimal stimulation parameters.[8] Based on these results, the present study was designed to verify the hypothesis that direct colonic stimulation can reduce mean transit time in a chronic porcine model 5 weeks after surgery. The local histological impact of the electrodes and stimulation on the caecal wall was also assessed.

## MATERIALS AND METHODS

Twelve healthy female farm pigs, weighing 50 ± 13 kg, were included. During the study, pigs were kept at the farm under standardized conditions with a regular feeding schedule. The protocol was approved by the local Ethics Committee on Animal Care (Office Vétérinaire Cantonal, Lausanne, Switzerland). All animals received care in compliance with the 'Principles of Laboratory Animal Care' formulated by the National Society for Medical Research and the 'Guide for the Care and Use of Laboratory Animals' (NIH Publication No. 86-23, revised 1985).

### Study design

For 6 days before surgery, pigs daily ingested a first course of radiopaque markers (Fig. 1). Reference colonic transit time (transit time 1) was then determined for each animal on the day of surgery. After electrodes implantation, pigs were allowed a 3-week recovery period. Animals were fed with a second course of markers during the fourth postoperative week, while performing sham stimulation twice daily. A second transit time (transit time 2) was then determined under anaesthesia to evaluate the impact of implanted material without stimulation. During the fifth postoperative week, a third course of markers was administered, while pigs were stimulated according to the protocol. After 6 days, a third transit time (transit time 3) was established and pigs were sacrificed with an intravenous overdose of pentobarbitone. Histological examination was performed on the caecal wall of six animals.

### Anaesthesia

Pigs were premedicated with 10 mg $kg^{-1}$ i.m. ketamin (Ketaminol; Vetarinaria AG, Zurich, Switzerland). Isoflurane 3–5% (Provet AG, Burgdorf, Switzerland) was administered via an air–oxygen mixture for induction. Then an oro-tracheal tube and intermittent positive pressure ventilation were used for the maintenance of general anaesthesia. Volume depletion was replaced with a continuous infusion of NaCl 0.9% solution during the operative period. An electrical warming cushion was used to keep central temperature at physiological level (39 ± 0.5 °C) monitored via a rectal thermometer. Analgesia was

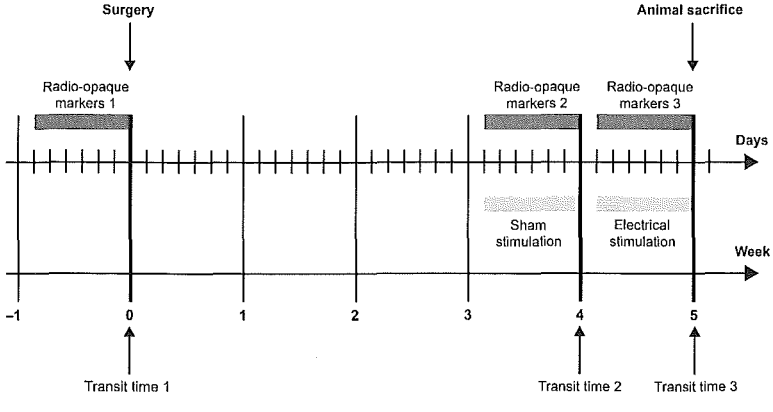


**Figure 1** Study design. Three courses of radiopaque markers were administered (grey boxes), allowing for the determination of the three colonic transit times. Day 0 corresponds to that of surgery. Sham and true stimulations are represented by dashed boxes.

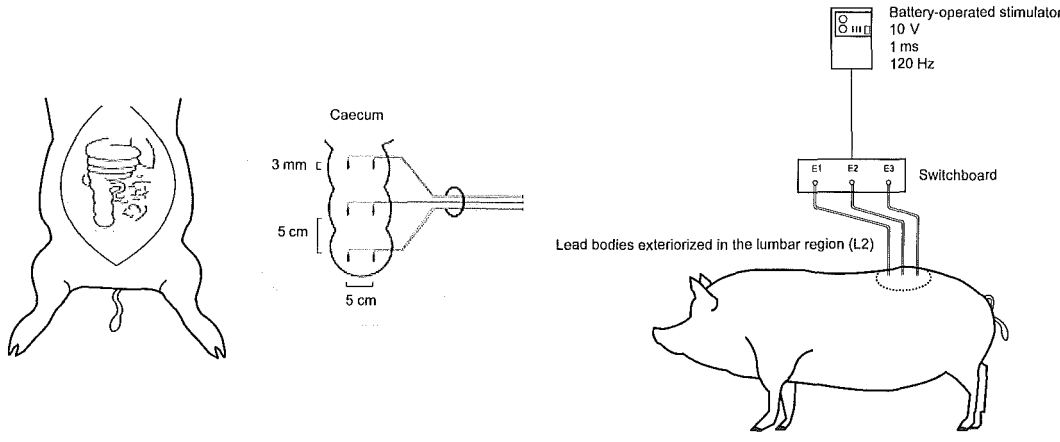


**Figure 2** On the left, pig's gastro-intestinal tract, with details of the caecum and the three pairs of longitudinally implanted electrodes. On the right, electrodes connected to a switchboard during sham and true stimulations. Electrical current was delivered by a battery operated device.

assured with 1 g i.v. paracetamol (Perfalgan; Upsamedica GmbH, Baar, Switzerland) and 100 $\mu$g i.v. bolus of fentanyl (Fentanyl-Janssen; Janssen-Cilag AG, Baar, Switzerland). Antibiotic prophylaxis was performed with 1 g i.v. cephazoline (Kefzol; Teva Pharma AG, Aesch, Switzerland) prior to incision.

## Surgical procedure and postoperative care

The caecum was exposed through a 15-cm long medial laparotomy, preserving mesenteric innervation and blood supply (Fig. 2). Caecal wall surface was kept moist with 39 °C sterile water. Three pairs of unipolar pacing electrodes (Medtronic Temporary Myocardial Pacing Lead 6500; Medtronic Inc., Minneapolis, MN, USA) were longitudinally implanted into the seromuscular layer, along both taenias, 5 cm apart. Electrodes are made of stainless steel with a length of 3 mm and a diameter of 0.8 mm. Final fixation was secured by knotting the non-conducting part of the electrodes to the caecal wall using a Mersilene 2-0 suture (Ethicon Inc., Somerville, NJ, USA). The lead body of the six electrodes was tunnelled subcutaneously from the peritoneal cavity to the lumbar region (L2 level). External wires were sutured to the skin with Vicryl 3-0 (Ethicon Inc.) and protected by sterile dressing. The laparotomy was closed using continuous Vicryl 0 suture for the deep muscular layer and separated Vicryl 3-0 stitches for the skin. A second dose of antibiotics, marbrofloxacine 10% (Marbrocyl; Vétoquinol S.A., Lure, France), was administered i.m..

Postoperative daily care included rectal temperature measurement and dressing evaluation. Pain was controlled using 1 g paracetamol orally (Dafalgan; Bristol-Myers Squibb, Baar, Switzerland) three times per day for 5 days postoperatively. Five millilitres i.m. phenylbutazone (Butadion ad us. vet.; G. Streuli & Cie. SA, Uznach, Switzerland) was also used for the first two postoperative days. Infection prophylaxis consisted in i.m. enrofloxacine 10% (Baytril; Bayer AG, Leverkusen, Deutschland) the first 2 days and then orally for eight more days. Animals were fed as usual with a supplemented crop mixture of barley, corn and oats.

## Colonic transit time measurement

For each of the three courses of radiopaque markers, colonic transit time was measured according to the technique currently used at our institution for the evaluation of colonic transit time and adapted from Chaussade *et al.*[9] Ten encapsulated markers (P. & A. Mauch, Münchenstein, Switzerland) were given daily at 9 AM for 6 days. A co-investigator was present to ensure the complete ingestion of the capsule. Plain abdominal X-rays were performed at 9 AM on the seventh day. The number of remaining markers was determined by two independent investigators allowing for calculation of colonic transit times.

## Electrical stimulation

Optimized stimulation parameters as defined in acute experiments by Aellen *et al.* were chosen.[8] Stimulations were performed on conscious pigs at the farm in standardized conditions (Fig. 2). Aboral sequential trains of 1-ms pulse width, 10 V amplitude and 120 Hz frequency were applied using an external battery operated stimulator (Screener 3625; Medtronic Inc., Minneapolis, MN, USA). Current was delivered during 20 s, starting with the most proximal pair of electrodes. The second and third pairs were then sequentially stimulated, in an aboral manner. After a 1-min stimulation sequence, a 1-min pause was observed. This protocol was repeated ten times in the morning and in the afternoon during the fifth postoperative week (Fig. 1). The same setup was used for sham stimulation on the fourth week, except that no electrical current was delivered.

## Histology

The postmortem obtained caecums were fixed in a 4% buffered formaline and were carefully examined by the surgeon and the pathologist. Samples were taken for histological examination along the electrode trajectory. The material was embedded in paraffin. Then 5-$\mu$m thick slides were performed and stained with Haematoxylin and Eosin.

## Statistical analysis

For each of the three courses of radiopaque markers, a mean colonic transit time value (MTT1, MTT2, MTT3) was computed from transit times obtained from each animal. They were compared using a Wilcoxon signed rank test for paired data. $P$ value $\leq 0.05$ was considered as statistically significant.

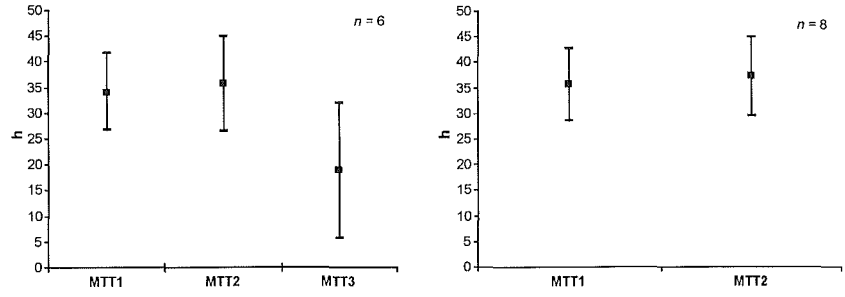


**Figure 3** On the left, evolution and comparison of MTT computed from transit times of each of the six pigs having completed the whole study protocol. Three phases were determined: before electrodes implantation (MTT1), after sham stimulation (MTT2) and after electrical stimulation (MTT3). MTT3 is significantly reduced when compared with MTT1 ($P = 0.045$) and MTT2 ($P = 0.035$). On the right, eight pigs obtained both transit times 1 and 2 which could be used for the computation and comparison of MTT1 and MTT2. No significant difference was observed between them ($P = 0.779$). MTT are represented with 95% confidence intervals.

## RESULTS

### Mean colonic transit time

Out of the 12 animals, transit times 1, 2 and 3 could be determined in eleven, nine and six animals respectively. Causes of withdrawal were related to wrong radiopaque administration (pig 2), postoperative gastric ulcer perforation (pig 3), electrode dislocation (pig 4–6) and laryngeal oedema (pig 7). Consequently, six pigs completed the whole study protocol. For those, MTT1 and MTT2 could be calculated and compared to MTT3 (Fig. 3). A statistically significant reduction between MTT1 (34 ± 7 h; mean ± SD) and MTT3 (19 ± 13 h) was found ($P = 0.045$). A significant reduction was also observed when comparing MTT2 (36 ± 9 h) to MTT3 ($P = 0.035$).

Both transit times 1 and 2 could be obtained in eight animals, all of which could be used for the computation and comparison of MTT1 and MTT2 (Fig. 3). No significant difference was observed between them (36 ± 9 and 37 ± 9 h respectively; $P = 0.779$).

### Histology

Two distinct histological patterns were identified depending on the position of the non-conducting parts of the electrodes in the caecal wall. Those with an intraluminal trajectory displayed an acute and dense inflammation composed of neutrophils, lymphocytes, histiocytes and fibrin along the electrodes (Fig. 4A). Caecal walls bearing exclusively intramural electrodes displayed a weak chronic inflammatory reaction, with moderate fibrosis, composed of histiocytes and lymphocytes with scattered giant cells (Fig. 4B). There was no acute peritonitis, although light peritoneal reaction was observed. The fixation

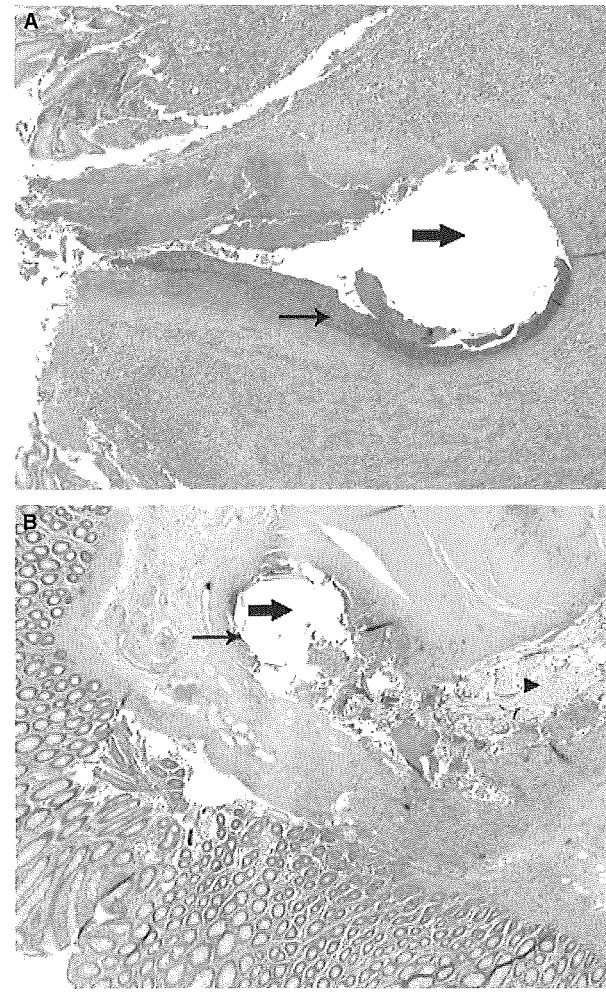


**Figure 4** (A) Intraluminal electrode course (thick arrow) with dense acute inflammatory reaction rich in neutrophiles and fibrin (thin arrow). In upper left corner, inflamed colonic mucosa is visible. H&E 40×. (B) Intramural electrode course (thick arrow) with weak chronic and giant cell inflammatory reaction (thin arrow). In bottom left corner, normal colonic mucosa is visible. Suture material is shown on the right (arrowhead). H&E 40×.

material induced a standard giant cell foreign body reaction.

## DISCUSSION

This study confirms that direct electrical colonic stimulation is feasible in a chronic animal model. Five weeks after electrodes implantation, a reduction of the mean colonic transit time was obtained after sequential colonic stimulation, with minimal parietal lesions.

Out of the twelve initially included animals, six were lost throughout the protocol. It resulted in wide confidence intervals for differences between MTT1 and MTT2, compared with MTT3. As this research was profiled as a preliminary study, active and sham stimulations were not randomized, constituting a limitation. Our results nevertheless provide evidence of the impact of direct electrical stimulation on the colon.

As previously reported, the pig is an adequate model to assess the colonic transit.[10,11] Davis *et al.* showed physiological transit times between 24 and 48 h.[10] In comparison, Ueda *et al.*, using radiopaque markers, observed longer transit times (57.4 ± 6 h).[11] In the present study, all transit times 1 and 2 were within 24–55 h. As porcine gastro-intestinal activity is influenced by age, alimentation, conditions of detention and other environmental factors attention must be paid to these variables when comparing data.[12] Other features confirm that the porcine model is appropriate. Its colonic anatomy, physiology and innervation, via the enteric nervous system, share large similarities with humans.[13,14]

In an attempt to translate animal research into clinical field, various constipated animal models have been used.[3,5] We did not choose such an approach because no matter which model, it does not correspond to the physiopathology of human constipation. The purpose of this study was to evaluate stimulation under the most physiological conditions, with standardized procedures. No invasive approach other than surgery, in particular colonoscopy, manometry or colonic preparations was used.

We chose the caecum for its accessibility and morphological aspects. Furthermore, previous experiments showed that the best colonic motor response was obtained with caecal stimulation.[8]

Three recent studies reported on the impact of direct colonic electrical stimulation.[5,6,8] Two acute experiments on pigs found that stimulation generated colonic contractions.[6,8] One chronic experiment on mongrel dogs showed an accelerated transit time under stimulation.[5] The present study confirms this last observation. The longest available pulse width (1 ms) was applied, considering the limitation of a commercial battery operated stimulator. Reduction of MTT was obtained 5 weeks after electrodes implantation. To our knowledge, it corresponds to the longest published time interval between surgery and stimulation.

Mean colonic transit time was significantly reduced, although only 10 cm of the proximal colon were sequentially stimulated in an aboral manner. It assumes an extended response, not limited to these 10 cm. Moreover, Dinning *et al.* have recently demonstrated that luminal content propulsion is highly dependent on colonic proximal activity.[15] Our electrical stimulation could have provoked a whole activation of the colon, either mechanically, by local contractions, or electrically, along its neuromuscular structure. This phenomenon should be studied in further experiments on local and distant bowel reactions to electrical stimulation. Studies should also focus onto the impact of electrical stimulation in the presence of an altered enteric nervous system and using an animal model of delayed colonic transit.[16]

Parietal impact of intramural electrodes and stimulation was limited to a minimal chronic inflammation and fibrosis at 5 weeks. Furthermore, the wires generated a peritoneal reaction. These phenomenons are routinely observed around implanted material. As this new technology might be used to stimulate the human colon, the development of more biocompatible material will therefore be mandatory.

Current conservative and surgical treatments of chronic constipation are disappointing and costly.[17–20] Electrical stimulation is actually under evaluation to treat various functional digestive disorders such as constipation.[6,21,22] Direct stimulation as performed by Shafik *et al.* is technically demanding and has never been duplicated by others.[7] Sacral neuromodulation represents a recent alternative via indirect sacral roots stimulation.[21,22] However, this technique requires intact neuronal pathways, has some anatomical contra-indications and its efficacy has never been demonstrated by randomized controlled trials.

The accessibility of the colon, the possibility to stimulate a specific segment and the innovative character of the presented technique open promising perspectives. Future research should focus onto the development of a minimally invasive implantable device designed for human application.

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## COMPETING INTERESTS

Nathalie Virag is a full time Medtronic Europe Sàrl employee and is a Medtronic stock owner. All other authors have no competing interests.

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