INTRODUCTION

Diabetes mellitus is a chronic, progressive condition that affects over 350 million people worldwide. It is one of the most prevalent chronic diseases, with around 1 million Australians currently diagnosed (Zheng, Ley, & Hu, 2018). The majority (90%) of diabetic patients suffer from the type 2 form of the disease, which is associated with poor diet, ageing, and genetic predisposition (Zheng et al., 2018). During early stages of type 2 diabetes, insulin-mediated glucose uptake in...
the liver, muscle, and adipose tissue becomes less effective, a state named insulin resistance. The high levels of insulin secreted in compensation lead to subsequent dysfunction of β-cells in the pancreas, which eventually leads to a permanent reduction in insulin secretion (Stumvoll, Goldstein, & van Haeften, 2005). The resulting high glycemic levels damage vital organs over time and can lead to serious complications such as the development of cardiovascular disease, retinopathy, and neuropathy (Zheng et al., 2018). A strategy for many therapies of type 2 diabetes is to modulate endogenous hormonal secretions from the pancreas to improve glycemic control in type 2 diabetes (Vetere, Choudhary, Burns, & Wagner, 2014).

Despite advancements in pharmacological therapies, up to half of type 2 diabetic patients fail to achieve adequate glycemic control due to poor medication adherence (Polonsky & Henry, 2016) or secondary failure of pharmacological treatments (Brown, Conner, & Nichols, 2010; Harrower, 1994). Medical adherence is worsened by the experience of unpleasant side effects, such as hypoglycemia (Polonsky & Henry, 2016; Walz et al., 2014), and with greater complexity of the medication administration regimen (Claxton, Cramer, & Pierce, 2001; Coleman et al., 2012; de Vries et al., 2014; Garcia-Perez, Alvarez, Dilla, Gil-Guillen, & Orozco-Beltran, 2013). Clinical management of type 2 diabetes usually involves a combination of drugs to improve glycemic control and each has its associated side effects. Antihyperglycemic drugs (biguanides) are associated with gastrointestinal dysfunctions such as diarrhea, nausea, and vomiting (Siavash, Tabbakhian, Sabzghabaei, & Razavi, 2017). Hypoglycemic drugs (sulfonylureas) are strongly associated with uncontrolled hypoglycemic episodes, which can lead to loss of consciousness, coma, and even death (Harrigan, Nathan, & Beattie, 2001; Spiller & Sawyer, 2006). This is also an issue with antihyperglycemic medication, where nearly a third of patients experience hypoglycemic side effects (Chao, Nau, & Aikens, 2007). Severely unpleasant side effects can lead to unreliable administration (i.e., skipping of doses) or even discontinuation of the therapy (Siavash et al., 2017). It is clear that a new therapy is needed to improve medical adherence, reduce secondary failure, and minimize side effects while avoiding hypoglycemic episodes. These are unmet clinical needs that cause suboptimal glycemic control and contribute to worsening of the disease.

Electrical stimulation of the vagus nerve has regulatory approval for the treatment of epilepsy, depression, and obesity (Payne, Furness, & Stebbing, 2019). However, the vagus nerve is also implicated in the regulation of energy metabolism, food intake, and glycemia as the nerve has a major role in the control of pancreatic hormonal secretions (Waise, Dranse, & Horowitz, 2014). Early studies in rats and dogs show that electrical stimulation of the vagus nerve affects the secretion of insulin and glucagon from the pancreas (Ahren, Paquette, & Taborsky, 1986; Ionescu, Rohner-Jeanrenaud, Berthoud, & Jeanrenaud, 1983; Nishi et al., 1987; Rozman, Bunc, & Zorko, 2004). However, the results of previous studies examining how vagus nerve stimulation regulates glycemia and glucose metabolism hormones have revealed conflicting results (Table 1). Vagus nerve stimulation delivered at low frequencies (5–30 Hz) activates both afferent fibers that send signals toward the brain, and efferent fibers that send signals toward the pancreas and other vital organs (Bonaz, Picq, Sinniger, Mayol, & Clarencon, 2013). Cervical vagus nerve stimulation at 5 Hz elevated fasting blood glucose in rats (Stauss, Stangl, Clark, Kwikte, & Lira, 2018). However, 30 Hz frequency abdominal stimulation reduced fasted blood glucose in obese mini-pigs (Malbert, Picq, Divoux, Henry, & Horowitz, 2017) and 5 Hz stimulation decreased glycemic response to an oral glucose tolerance test type 2 diabetic rats (Yin, Ji, Gharibani, & Chen, 2019). An important distinction in the role of afferent and efferent vagal signaling was evidenced by stimulating only the proximal or distal cut end of the cervical rat vagus nerve at 5 Hz, which resulted in an afferent-driven increase in glycemia or an efferent-driven decrease in glycemia due to corresponding changes in pancreatic secretions (Meyers, Kronemberger, Lira, Rahmouni, & Stauss, 2016). In a different approach, high-frequency stimulation in the kilohertz range is presumed to block nerve activity (Camilleri et al., 2008; Tweden, Anvari, et al., 2006; Tweden, Sarr, et al., 2006). Stimulation at 5 kHz is thought to block afferent vagal ‘hunger’ signaling to the brain and is used clinically for the treatment of obesity (Camilleri et al., 2008; Payne, Furness, & Stebbing, 2019), but also reduces fasted glucose levels and glycated hemoglobin in diabetic patients (Shikora et al., 2013). As such, a better understanding of the effect of vagus nerve stimulation on glycemic levels and underlying hormone secretions is an important first step in the development of new treatments of the regulation of glycemia in type 2 diabetes.

To date, there is no one comparative study that assesses the effects of various electrical stimulation strategies of the abdominal vagus nerve on glycemia and pancreatic secretions (Table 1). Furthermore, a notable absence in these previous studies is a report of how the incretin hormone GLP-1 is affected by stimulation (Table 1). Incretin hormones are released from the small intestine to trigger the additional release of insulin during postprandial phases (Berthoud, 2008) and can be affected by electrical stimulation of the vagus nerve (Rocca & Brubaker, 1999). As such, here we will examine changes in the levels of glucose, insulin, glucagon, and GLP-1 during the application of: (a) low-frequency 15 Hz stimulation (‘VNS’), designed to increase activity in the vagus nerve; (b) a combination of low (15 Hz)- and high (40 kHz)-frequency stimulation, designed to provide ‘directional’ activation in which afferent (‘aVNS’) or efferent (‘eVNS’) fibers are primarily activated (Patel, Saxena, Bellamkonda, & Butera, 2017); (c) high-frequency 40 kHz stimulation, in which kilohertz frequency alternating current
is applied to produce a focal block on the nerve and inhibit activity (Kilgore & Bhadra, 2014), and high-frequency ‘4 kHz stimulation, which mimicked the commercially available “vBloc maestro” stimulation strategy for the treatment of obesity’ (Camilleri et al., 2008; Ikramuddin et al., 2014). As such, this study seeks to determine the effects of various vagus nerve stimulation strategies for modulating glycemia and provide a comparative study with the aim to establish a basis for clinical translation.

# 2 | MATERIALS AND METHODS

## 2.1 | Animals and anesthesia

All experiments used male Sprague–Dawley rats (8–10 weeks, Animal Resource Centre, Western Australia). Procedures were approved by the Bionics Institute Animal Research Ethics Committee (17-369AB, 18-384AB) and complied with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council of Australia) and the Prevention of Cruelty to Animals (1986) Act. Animals were kept on a 12 hr light/dark cycle and allowed access to standard chow and water ad libitum. Rats were fasted overnight (14–16 hr) and anesthetized (1.5%–2% isoflurane in oxygen flowing at 1 L/min) prior to the terminal surgical procedure. Breathing rate was maintained between 45 and 60 breaths per minute for the duration of the nonrecovery experiment. At the conclusion of the experiment, rats were euthanized (300 mg/kg Lethabarb, intravenous injection).

## 2.2 | Vagus nerve electrode array

The vagus nerve electrode array consisted of six platinum (99.95%) electrodes embedded into a medical-grade silicone-elastomer cuff. Each platinum electrode had an exposed surface area of 0.39 mm². The electrodes were arranged in pairs, on opposite sides of the cuff, and with a distance of 3.4 mm between electrode pairs (Figure 1a). A rectangular-section

### TABLE 1  Effects of vagus nerve stimulation on glycemia and pancreatic hormone secretions

| Reference                | Species/model | Stim parameters | Stim site                                      | Glucose | Insulin | Glucagon | GLP-1 |
|--------------------------|---------------|----------------|-----------------------------------------------|---------|---------|----------|--------|
| Ionescu et al. (1983)    | Rat/normal    | 30 Hz, 50 µA, 0.2 ms | Dorsal motor nucleus                         | ↑       | ↑       | -        | -      |
| Ahren et al. (1986)      | Dog/normal    | 10 Hz, 13.5 mA, 5 ms | Anterior and posterior thoracic vagus nerves | ↑       | ↑       | ↑        | -      |
| Nishi et al. (1987)      | Rat/normal    | 10 Hz, 10 V, 1 ms | Subdiaphragmatic anterior and posterior vagus nerves | -       | ↑       | ↑        | -      |
| Rozman et al. (2004)     | Dog/normal Alloxan | 20 Hz, 1 mA, 200 µs | Cervical vagus nerve                         | -       | ↑       | ↑        | -      |
| Meyers et al. (2016)     | Rat/normal    | 5 Hz, 3 V, 1 ms VNS aVNS eVNS | Right or left cervical vagus nerve | ↑ = = | = = | ↑ = | - |
| Stauss et al. (2018)     | Rats/normal   | 5 Hz, 3 V, 1 ms| Right cervical vagus nerve                    | ↑       | ↓       | =        | -      |
| This study               | Rat/normal    | 15 Hz VNS aVNS eVNS | Subdiaphragmatic anterior vagus nerve         | ↑ = = | = = | ↑ = | = = = |
| Yin et al. (2019)        | Rats/normal T2DM | 5 Hz, 2 mA, 0.3 ms | Subdiaphragmatic anterior vagus nerve        | ↓ = | - | = | - |
| Malbert et al. (2017)    | Mini pigs/obese | 30 Hz | Subdiaphragmatic anterior and posterior vagus nerves | ↓ = | - | = | - |
| Shikora et al. (2013)    | Human/obese & T2DM | 5 kHz, 3–8 mA | Anterior and posterior gastric vagus nerves | ↓ = | - | - | - |

*Experiment stimulated the cut proximal and distal ends of the cervical vagus nerve. Arrows (↑ ↓) indicate a significant increase or decrease (p > .05), an equal sign (=) has no statistically significant change (p ≥ .05), and a dash (-) indicates data were not assessed.
lumen (0.55 mm × 0.2 mm) traversed the cuff for housing the vagus nerve. The silicone cuff was sutured closed to prevent the nerve from migrating out of the cuff. A silicone suturing tab on the lead was used to anchor the array to the esophagus to provide mechanical stability. A helical cable with 25-µm-diameter platinum/iridium (90/10) wires from the electrodes was tunneled to a percutaneous connector mounted on the lumbar region of the rat.

### 2.3 Surgical interventions

In brief, the ventral abdominal midline was incised and the ventral esophagus and subdiaphragmatic anterior abdominal branch of the vagus nerve exposed, similar to that done previously (Payne, Furness, Burns, et al., 2019). The nerve was dissected away from the esophagus and the array implanted rostral to the hepatic and celiac branches of the vagus. The array was sutured (7-0 silk, Ethicon) to the esophagus to provide stabilization and the abdominal cavity and skin sutured closed. As a method to withdraw venous blood and deliver the glucose bolus, the left and right femoral veins were exposed and cannulated. The cannula lines were kept patent during the experiment by using sterile saline and heparin (10 units/ml). Animals were kept hydrated and blood loss replaced with similar volumes of sterile Hartman’s solution. Surgical implantation of the electrode cuff on the abdominal vagus was successful in all the 33 naïve animals used. Two animals were excluded from the experimental dataset due to anesthetic issues and one due to a diabetic-type response during the initial glucose tolerance test (blood glucose > 30 mM sustained more than 2 hr after an intravenous (i.v.) bolus).

### 2.4 Glucose tolerance test

At $T = -5$ min, control baseline blood samples were taken. An intravenous bolus of glucose (500 mg/kg) was administered at $T = 0$, and a series of blood samples were taken at $T = 5, 12, 30, 60,$ and $90$ min (Figure 1b). Vagus nerve stimulation (details provided below) was applied for 60 min immediately following intravenous glucose bolus (indicated by the syringe icon). In test 2 (T2), the intervention applied was interleaved with a no-stimulation control.

### 2.5 Electrode impedance testing and electrophysiological recordings

Functionality of electrodes was tested by measuring the impedance from voltage transients to current pulses (25 µs,
100 µA), in common-ground configuration (one active electrode vs. all others as return) as was reported previously (Fallon, Irvine, & Shepherd, 2009). Electrically evoked compound action potentials (ECAPs) were recorded to determine threshold of neural activation. ECAPs were generated by stimulating the proximal electrode pair (200 µs; 15 Hz; 0–2 mA) and recording from the distal pair (averaged over 100 pulses and repeated twice), closest to the pancreas. Recordings were sampled at a rate of 200 kHz and digitally filtered (20–2,000 Hz band pass; Payne, Furness, Burns, et al., 2019). The ECAP threshold was defined as the minimum stimulus intensity producing a response amplitude of at least 2 µV within a poststimulus latency window of 4–10 ms. This latency corresponds to conduction velocities within the range of C-fiber responses (Castoro et al., 2011). In all experiments the stimulation current was suprathreshold (detailed in the next section).

2.6 Vagus nerve stimulation strategies

A custom-made external stimulator was used to deliver biphasic current pulses at minimum 15 Hz and up to 4 kHz (Fallon et al., 2018). For even higher-frequency stimulation, a 40 kHz sinusoid waveform was output from a function generator and isolated via a transformer. The following stimulation strategies were used: (a) Low-frequency stimulation, referred to as ‘VNS’, applied at 15 Hz, 200 µs biphasic square pulses at 1 mA (Figure 2a); (b) High-frequency 40 kHz stimulation, referred to as ‘40 kHz’, applied at 40 kHz sinusoidal frequency and a current of 4 mA (peak to peak; Figure 2a); (c) High-frequency 4 kHz stimulation, referred to as ‘4 kHz’, applied 4 kHz of 100 µs, 2 mA, biphasic square pulses (Figure 2a); (d) Directional afferent stimulation, referred to as ‘aVNS’, simultaneously applied 40 kHz sinusoidal current of 4 mA (peak to peak) to the central electrode pair and 15 Hz, 200 µs square biphasic pulses to the proximal electrode pair (Figures 1a and 2a). Current level applied was within the ‘therapeutic blocking window’ (see below for details) and; (e) directional efferent stimulation, referred to as ‘eVNS’, simultaneously applied 40 kHz sinusoidal current of 4 mA (peak to peak) to the central electrode pair and 15 Hz, 200 µs square biphasic pulses to the distal electrode pair (Figures 1a and 2a,b1,b2). Current level applied was within the ‘therapeutic blocking window’ (see next paragraph for details; Figure 2b1,b2).
2.7 | Therapeutic blocking window

The therapeutic blocking window was determined by assessing the neural response threshold when blocking was applied (Figure 2b2), compared to when no blocking is applied (Figure 2b1). Application of 40 kHz stimulation to the nerve was only effective in blocking the nerve activity induced by 15 Hz stimulation below a certain current level. The ‘therapeutic blocking window’ refers to the range of current levels that elicited nerve activity at 15 Hz, but where the activity was not observed when combined with 40 kHz stimulation, to remain efficient at blocking the nerve in one direction. The example in Figure 2b shows the neural threshold is 150 µA (Figure 2b1) during no blocking, but increased to 300 µA when 40 kHz stimulation was used to block afferent activity (Figure 2b2). Therefore, in this example applying 15 Hz and a current level higher than 150 µA but lower than 300 µA (i.e., the therapeutic blocking window) will be effective in directionally activating the nerve. The therapeutic blocking threshold is determined separately in each subject, as the threshold will differ between rats.

2.8 | Quantification of glycemia and hormones

Whole blood samples (300 µl) from the femoral vein were collected in K2-EDTA coated tubes (Starstedt). Glycemia was measured using a glucometer (Accu-Chek Nano, Roche), and the remaining blood was centrifuged (2,000 g for 10 min), plasma aliquoted, and stored at −80°C. To validate the use of the glucometer used (Accu-Chek Nano, Roche), a subset of plasma samples was compared with a glucose assay (kit 81693, Crystal Chem) and were found to be highly correlated (Pearson $R^2 = .85$). On the day of the assay, aliquots were thawed and enzyme-linked immuno-sorbent assays (ELISA) were performed according to manufacturer's instructions (Crystal Chem Inc) for insulin (kit: 90010), glucagon (kit: 81519), and GLP-1 (kit: 81507). The hormone levels were determined via absorbance measurements using a FLUOStar Omega kinetic plate reader (BMG Labtech). For each glucose or hormone response, the baseline at $T = -5$ min was subtracted and the area under the curve calculated over the 60-min period during which stimulation was applied.

2.9 | Statistical analysis

The net effect of vagus nerve stimulation on the levels of glucose, glucagon, insulin, and GLP-1 over the 60-min testing period was compared to unstimulated response within the same rat using paired student $t$ tests to assess differences. Statistically significant differences were accepted as $p < .05$ and GraphPad was Prism 4 (GraphPad Software) used for all analysis. Values are reported as mean ± standard error of the mean.

3 | RESULTS

3.1 | Impedance of implanted electrodes and electrically evoked neural responses

The impedance of electrodes in vitro (saline) was 4.5 ± 0.4 kΩ, while the impedance of the electrodes in vivo was 6.3 ± 2 kΩ. ECAPs were recorded to ensure stimulation levels remained above threshold. The mean threshold for evoking ECAP responses was 692 ± 406 µA. There were no changes in neural thresholds following the 60-min application of 4 kHz or 40 kHz stimulation.

3.2 | Effects of low-frequency vagus nerve stimulation

Low-frequency 15 Hz stimulation produced a significant increase in glucose levels of +2.9 ± 0.23 mM·hr (+47%, paired $t$ test $p = .015; n = 5$), a significant increase in glucagon of +17.1 ± 8.05 pg·hr/ml (+1,454%, $p = .022$), and no statistically significant changes in insulin (−0.29 ± 0.24 ng·hr/ml; −75%; $p = .11$) and GLP-1 levels (−0.4 ± 1.31 pg·hr/ml; −342%; $p = .12$; Figure 3b,d,f,g).

3.3 | Effects of high-frequency vagus nerve stimulation

Application of 4 kHz and 40 kHz stimulation strategies had different effects on glucose and glucagon levels. Stimulation at 4 kHz ($n = 5$) caused a significant increase in glucose levels (+1.5 ± 0.5 mM·hr; +26% $p = .049$) that was accompanied by non-statistically-significant changes in glucagon (+1.1 ± 0.14 pg·hr/ml; +19%; $p = .47$), insulin (−0.08 ± 0.061 ng·hr/ml; −9%; $p = .463$), and GLP-1 levels (+1.0 ± 1.1 pg·hr/ml; +153%; $p = .251$, Figure 3b,d,f,h). However, application of 40 kHz ($n = 3$) resulted in a statistically significant decrease in glucagon levels (−12.3 ± 3.2 pg·hr/ml; −249%; $p = .009$, Figure 3c,d). There were no statistically significant changes in glucose (+0.5 ± 0.49 mM·hr; +13%; $p = .445$), insulin (+0.20 ± 0.22 ng·hr/ml; +33%; $p = .463$ Figure 3e,f), and GLP-1 levels (−1.20 ± 0.74 pg·hr/ml; −262%; $p = .25$ during 40 kHz stimulation.
3.4 Effects of directional nerve stimulation

A contrast was seen between the effects of afferent VNS \((n = 7)\) and efferent VNS \((n = 9)\) stimulation strategies on glucose and hormone levels. Application of aVNS produced a large and statistically significant increase in glucose levels of \(+2.4 \pm 1.5 \text{ mM-hr} \) (63%; \(p < .01\)), which was accompanied by a significant increase in glucagon levels \(+39 \pm 15 \text{ pg-hr/ml} \) (485%; \(p = .04\)). There were no statistically significant changes in insulin \(+0.10 \pm 0.26 \text{ ng-hr/ml} \) (17%; \(p = .31\)) and GLP-1 secretions \(+1.1 \pm 1.64 \text{ pg-hr/ml} \) (332%; \(p = .282\); Figure 3b,d,f,h). In contrast, eVNS produced no statistically significant changes in glucose \(+0.26 \pm 0.38 \text{ mM-hr} \) (6%; \(p = .80\); Figure 3a,b), glucagon \(-1.4 \pm 11.9 \text{ pg-hr/ml} \) (10%; \(p = .944\)), insulin \(-0.10 \pm 0.2 \text{ ng-hr/ml} \) (13%; \(p = .31\)) and GLP-1 levels \(-1.0 \pm 0.5 \text{ pg-hr/ml} \) (186%; \(p = .130\); Figure 3b,d,f,h).

4 DISCUSSION

The vagus nerve regulates energy metabolism, food intake, and glycemic control. Here, we found that applying different electrical stimulation strategies to the abdominal (anterior)
vagus nerve modulates glycemia by effecting glucagon and insulin secretions. In particular, applying high-frequency 40 kHz stimulation lowered glucagon secretions and may have a potential application for developing new treatments of type 2 diabetes.

The anterior abdominal vagus nerve resides below vagal branches to heart and lungs and avoids unwanted off-target activation, and allows for the application of high levels of stimulation that may not be tolerated when applied to the cervical vagus nerve (Payne et al., 2018; Payne, Furness, Burns, et al., 2019; Payne, Furness, & Stebbing, 2019). Stimulation of the anterior subdiaphragmatic (abdominal) vagus nerve has previously been shown to decrease the glycemic response to an oral glucose tolerance test in type 2 diabetic rats (Yin et al., 2019). In rats, the majority of fibers from the left cervical vagus nerve form the anterior abdominal vagus, which branches into hepatic and celiac (and gastric) nerves (Berthoud & Neuhuber, 2000). The hepatic nerve innervates the liver, which has a major role in glucose homeostasis, and the head of the pancreas and the proximal duodenum (containing GLP-producing intestinal cells; Berthoud & Neuhuber, 2000; Phillips, Baronowsky, & Powley, 1997), while the distal pancreatic lobe receives sparse innervation from the celiac nerve (Berthoud, Carlson, & Powley, 1991; Bockman, 1993; Teff and Teff, 2008). Although there are differences in the parasympathetic neural innervation of the human pancreas, electrical stimulation of cholinergic fibers significantly increased insulin sections in an isolated human pancreas model (Brunicardi, 1995).

Low-frequency 15 Hz vagus nerve stimulation likely activated both afferent and efferent fibers, and caused a substantial increase in glycemia compared to unstimulated animals during a glucose challenge test. The hyperglycemic effect can be attributed to the activation of afferent vagal fibers, as a similar hyperglycemic response was observed during directional afferent, but not efferent, stimulation. Haptic vagal afferents reportedly act as a glucose sensor, as the mean rate of haptic neural activity decreased following an injection of glucagon to induce hyperglycemia, but increased in response to insulin-induced hypoglycemia (Niiijima, 1982, 1983). As such, electrically driven increases in vagal afferent activity may simulate a hypoglycemic state that leads to a feedback controlled increase in glycemia. Surprisingly, high-frequency stimulation at 4 kHz, which is presumed to block the firing of afferent and efferent vagal fibers in humans (Camilleri et al., 2008, 2009), also resulted in hyperglycemia. However, here we suggest that applying 4 kHz was ineffective at blocking neural activity, and in instead promoted an increase in afferent fiber activity, which lead to the hyperglycemic effect observed in our experiments.

Glucagon levels were affected by abdominal vagus nerve stimulation. When circulating levels of glucose in the blood stream become low, glucagon is released by α-cells of the islet of Langerhans in the pancreas which stimulates the release of glucose in the liver in a process known as gluconeogenesis (Miller et al., 2013). Applying 15 Hz vagus nerve stimulation and afferent stimulation resulted in a substantial increase in glucagon levels. This finding is in line with the theory that electrically increasing the activity of afferent vagal fibers simulates an ‘artificial’ hypoglycemic state and causes the increased release of glucagon, and subsequently leads to hyperglycemia (Niiijima, 1982, 1983).

Concurrently, inhibiting vagal (i.e., afferent) activity by applying 40 kHz stimulation leads to a substantial decrease in glucagon secretions. This is an important finding and may have application in the treatment of type 2 diabetes. Biguanides (brands include: Metformin, Diabex and Diaformin) are the most common class of medication used to control hyperglycemia of type 2 diabetes, and work by inhibiting glucose release from the liver by suppressing hepatic glucagon signaling (Miller et al., 2013). As such, glucagon-lowering effects of the 40 kHz blocking stimulation strategy may potentially have useful application to the treatment of type 2 diabetes.

Previous studies demonstrate an increase in insulin levels (Table 1) in response to hyperglycemic levels when VNS is applied (Ahren et al., 1986; Ionescu et al., 1983). In this study no statistically significant changes in insulin were seen during the application of any electrical stimulation strategy, despite the substantial hyperglycemic effect seen during the application of VNS, aVNS, and 4 kHz stimulation strategies. However, significant hyperglycemia in the absence of insulin release during low-frequency vagus nerve stimulation has been reportedly previously (Meyers et al., 2016), and is thought to be due to afferent-dependent suppression of insulin release that is centrally mediated by the indirect activation of sympathetic splanchnic nerve, known to cause suppression of insulin release (Andersson et al., 1982; Dunning, Ahren, Veith, & Taborsky, 1988; Holst, Schwartz, Knuhtsen, Jensen, & Nielsen, 1986; Meyers et al., 2016).

GLP-1 is another hormone that regulates glucose metabolism by promoting the release of insulin during post-prandial phases of ingestion, and is a therapeutic target of type 2 diabetes medications. The vagus nerve extends branches into the gastrointestinal tract to innervate K- and L-cells which produce the ‘incretin’ hormone GLP-1 following ingestion of glucose (Berthoud, 2008). GLP-1 promotes the release of insulin during postprandial phases of ingestion, and is a therapeutic target of a class of type 2 diabetic medications (GLP-1 agonists). Although a previous study showed that low-frequency electrical stimulation (20 Hz, 10 V) of the celiac vagal branch increased secretions of GLP-1 (Rocca & Brubaker, 1999), in the current study we found inconsistent changes in the secretion of GLP-1 during the application of different stimulation strategies. The difference between studies might be explained by the fact that the posterior celiac vagus nerve supplies the
small and large intestine, whereas the nerve terminals of the anterior celiac nerve only supply as far as the duodenum (in rat) (Berthoud et al., 1991). However, in this study the left or anterior abdominal vagus nerve was stimulated (15 Hz). As such, stimulation of both the anterior and posterior vagus nerves could lead to an increase in GLP-1 levels and provide potential therapeutic effect of decreasing glycemia.

A previous study achieved selective activation of cervical vagal afferent or efferent fibers by stimulating the cut proximal or distal end of the vagus nerve (Meyers et al., 2016). In this study, we achieved reversible directional stimulation by combining kilohertz-frequency blocking with low-frequency activation (Patel et al., 2017), and applied it to an abdominal location of the vagus nerve, close to the end organ. This is the first study that applied directional stimulation in order to control glycemia and hormone levels, and our technology allows for the long-term assessment of this stimulation strategy in awake animals. Future studies should consider the effects of these stimulation strategies in a rat model of type 2 diabetes.

Inhalant isoflurane anesthesia was used in this study to enable long experimental testing to be conducted to evaluate the effects of different stimulation strategies. Isoflurane reportedly inhibits the release of insulin from the pancreas and impairs glucose tolerance and clearance in humans (Dittoer & Camu, 1988; Tanaka, Nabatame, & Tanifuji, 2005). Our results are consistent with a previous study that shows no increases in insulin secretions during elevated glucose levels induced by VNS in isoflurane-anesthetized rats (Meyers et al., 2016). Furthermore, the suppression of insulin has been reported during application of VNS in awake rats, which resulted in elevated fasted blood glucose levels and an impaired glucose tolerance (Stauss et al., 2018). However, to remove the potential confounding effects of isoflurane, future studies should consider assessing the effect of various VNS stimulation strategies in awake animals.

In conclusion, electrical stimulation of the abdominal vagus nerve modulates glycemia by effecting glucagon secretions. Application of high-frequency 40 kHz stimulation, which lowered glucagon levels, may have a potential application for the treatment of type 2 diabetes.

ETHICS STATEMENT
Procedures were approved by the Bionics Institute Animal Research Ethics Committee (17-369AB, 18-384AB) and complied with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council of Australia) and the Prevention of Cruelty to Animals (1986) Act.

ACKNOWLEDGMENTS
We would like to acknowledge Ms Amy Morley and Ms Judy Gooley for molecular assistance.

CONFLICT OF INTEREST
All authors declare no conflicts of interest, financial or otherwise.

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REFERENCES
Ahren, B., Paquette, T. L., & Taborsky Jr., G. J. (1986). Effect and mechanism of vagal nerve stimulation on somatostatin secretion in dogs. American Journal of Physiology, 250, E212–217. https://doi.org/10.1152/ajpendo.1986.250.2.E212
Andersson, P. O., Holst, J., & Jarhult, J. (1982). Effects of adrenergic blockade on the release of insulin, glucagon and somatostatin from the pancreas in response to splanchnic nerve stimulation in cats. Acta Physiologica Scandinavica, 116, 403–409. https://doi.org/10.1111/j.1748-1716.1982.tb07158.x
Berthoud, H. R. (2008). The vagus nerve, food intake and obesity. Regulatory Peptides, 149, 15–25. https://doi.org/10.1016/j.regpep.2007.08.024
Berthoud, H. R., Carlson, N. R., & Powley, T. L. (1991). Topography of efferent vagal innervation of the rat gastrointestinal tract. American Journal of Physiology, 260, R200–207. https://doi.org/10.1152/ajpregu.1991.260.1.R200
Berthoud, H. R., & Neuhuber, W. L. (2000). Functional and chemical anatomy of the afferent vagal system. Autonomic Neuroscience, 85, 1–17. https://doi.org/10.1016/S1566-0702(00)00215-0
Bockman, D. E. (1993). Anatomy of the pancreas. The pancreas: Biology, pathobiology and disease.
Bonaz, B., Picq, C., Sinniger, V., Mayol, J. F., & Claneccon, D. (2013). Vagus nerve stimulation: From epilepsy to the cholinergic anti-inflammatory pathway. Neurogastroenterology and Motility, 25, 208–221. https://doi.org/10.1111/nmo.12076
Brown, J. B., Conner, C., & Nichols, G. A. (2010). Secondary failure of metformin monotherapy in clinical practice. Diabetes Care, 33, 501–506. https://doi.org/10.2337/dc09-1749
Brunicardi, F. C. (1995). Neural regulation of the endocrine pancreas. International Journal of Gastrointestinal Cancer, 18, 177.
Camilleri, M., Touli, J., Herrera, M. F., Kow, L., Pantoja, J. P., Billington, C. J., ... Moody, F. G. (2009). Selection of electrical algorithms to treat obesity with intermittent vagal block using an implantable medical device. Surgery for Obesity and Related Diseases, 5(2), 224–229. https://doi.org/10.1016/j.soard.2008.09.006
Camilleri, M., Touli, J., Herrera, M. F., Kulseng, B., Kow, L., Pantoja, J. P., ... Anvari, M. (2008). Intra-abdominal vagal blocking (vBloc therapy): Clinical results with a new implantable medical device. Surgery, 143, 723–731. https://doi.org/10.1016/j.surg.2008.03.015
Castoro, M. A., Yoo, P. B., Hincapie, J. G., Hamann, J. J., Ruble, S. B., Wolf, P. D., & Grill, W. M. (2011). Excitation properties of the right cervical vagus nerve in adult dogs. Experimental Neurology, 227, 62–68. https://doi.org/10.1016/j.expneurol.2010.09.011

Chao, J., Nau, D. P., & Aikens, J. E. (2007). Patient-reported perceptions of side effects of antihyperglycemic medication and adherence to medication regimens in persons with diabetes mellitus. Clinical Therapeutics, 29, 177–180. https://doi.org/10.1016/j.clinthera.2007.01.014

Claxton, A. J., Cramer, J., & Pierce, C. (2001). A systematic review of the associations between dose regimens and medication compliance. Clinical Therapeutics, 23, 1296–1310. https://doi.org/10.1016/S0142-9818(01)80109-0

Coleman, C. I., Limone, B., Sobieraj, D. M., Lee, S., Roberts, M. S., Kaur, R., & Alam, T. (2012). Dosing frequency and medication adherence in chronic disease. Journal of Managed Care Pharmacy, 18, 527–539. https://doi.org/10.18553/jmcp.2012.18.7.527

de Vries, S. T., Keers, J. C., Visser, R., de Zeeuw, D., Haaijer-Ruskamp, M. F., Voorham, J., & Denig, P. (2014). Medication beliefs, treatment complexity, and non-adherence to different drug classes in patients with type 2 diabetes. Journal of Psychosomatic Research, 76, 134–138. https://doi.org/10.1016/j.jpsychores.2013.11.003

Diltoer, M., & Camu, F. (1988). Glucose homeostasis and insulin secretion during isoflurane anesthesia in humans. Anesthesiology, 68, 880–886. https://doi.org/10.1097/00000542-198806000-00008

Dunning, B. E., Ahren, B., Veith, R. C., & Taborsky Jr., G. J. (1988). Nonadrenergic sympathetic neural influences on basal pancreatic hormone secretion. American Journal of Physiology, 255, E785–792. https://doi.org/10.1152/ajpendo.1988.255.6.E785

Fallon, J. B., Irvine, D. R., & Shepherd, R. K. (2009). Cochlear implant use following neonatal deafness influences the cochleotopic organization of the primary auditory cortex in cats. The Journal of Comparative Neurology, 512, 101–114. https://doi.org/10.1002/cne.21886

Fallon, J. B., Senn, P., & Thompson, A. C. (2018). A highly configurable neurostimulator for chronic pre-clinical stimulation studies. In Proceedings of the Neural Interfaces Conferences; June 25-27, 2018, Minneapolis, MN. Retrieved from https://www.neuralinterfaceconference.org/

Garcia-Perez, L. E., Alvarez, M., Dilla, T., Gil-Guillen, V., & Orozco-Dunning, B. E., Ahren, B., Veith, R. C., & Taborsky Jr., G. J. (1988). Obesity-associated alterations in glucose metabolism are reversed by chronic bilateral stimulation of the abdominal vagus nerve. Diabetes, 66, 848–857. https://doi.org/10.2337/db16-0847

Garcia-Perez, L. E., guardia, B. E., Ahren, B., Veith, R. C., & Taborsky Jr., G. J. (1988). Glucose homeostasis and insulin secretion during isoflurane anesthesia in humans. Anesthesiology, 68, 880–886. https://doi.org/10.1097/00000542-198806000-00008

Ionescu, E., Rohner-Jeanrenaud, F., Berthoud, H. R., & Jeanrenaud, B. (1983). Increases in plasma insulin levels in response to electrical stimulation of the dorsal motor nucleus of the vagus nerve. Endocrinology, 112, 904–910. https://doi.org/10.1210/endo-112-3-904

Kilgore, K. L., & Bhadra, N. (2014). Reversible nerve conduction block using kilohertz frequency alternating current. Neuromodulation, 17, 242–254.

Malbert, C. H., Picq, C., Divoux, J. L., Henry, C., & Horowitz, M. (2017). Obesitas-associated alterations in glucose metabolism are reversed by chronic bilateral stimulation of the abdominal vagus nerve. Diabetes, 66, 848–857. https://doi.org/10.2337/db16-0847

Meyers, E. E., Kronemberger, A., Lira, V., Rahmouni, K., & Stauss, H. M. (2016). Contrast effects of afferent and efferent vagal nerve stimulation on insulin secretion and blood glucose regulation. Physiological Reports, 4. https://doi.org/10.14814/phy2.12718

Miller, R. A., Chu, Q., Xie, J., Foretz, M., Viollet, B., & Birnbaum, M. J. (2013). Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature, 494, 256–260. https://doi.org/10.1038/nature11808

Niijima, A. (1982). Glucose-sensitive afferent nerve fibres in the hepatic branch of the vagus nerve in the guinea-pig. Journal of Physiology, 332, 315–323. https://doi.org/10.1113/physiol.1982.sp014415

Niijima, A. (1983). Glucose-sensitive afferent nerve fibers in the liver and their role in food intake and blood glucose regulation. Journal of the Autonomic Nervous System, 9, 207–220. https://doi.org/10.1016/0165-1838(83)90142-X

Payne, S. C., Furness, J. B., Burns, O., Sedo, A., Thomas, R., de Silva, A., Sedo, A., … Shepherd, R. K. (2018). Vagus nerve stimulation to treat inflammatory bowel disease: A chronic, preclinical safety study in sheep. Bioelectronics in Medicine, 1(4), 235–250. https://doi.org/10.2217/bem-2018-0011

Polonsky, W. H., & Henry, R. R. (2016). Poor medication adherence in patients with type 2 diabetes. Journal of Psychosomatic Research, 79, 207–217. https://doi.org/10.1016/j.jpsychres.2015.11.003

Phillips, R. J., Baronowsky, E. A., & Powley, T. L. (1997). Afferent innervation of gastrointestinal tract smooth muscle by the hepatic branch of the vagus. The Journal of Comparative Neurology, 384(2), 248–270. https://doi.org/10.1002/(SICI)1096-9861(19970728)384:2<248:AID-CNENB3.0.CO;2-i

Polonsky, W. H., & Henry, R. R. (2016). Poor medication adherence in type 2 diabetes: Recognizing the scope of the problem and its key contributors. Patient Preference and Adherence, 10, 1299–1307.
Rocca, A. S., & Brubaker, P. L. (1999). Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology, 140*, 1687–1694. https://doi.org/10.1210/endo.140.4.6643

Rozman, J., Bunc, M., & Zorko, B. (2004). Modulation of hormone secretion by functional electrical stimulation of the intact and incompletely dysfunctional dog pancreas. *Brazilian Journal of Medical and Biological Research, 37*, 363–370. https://doi.org/10.1590/S0100-879X2004000300012

Shikora, S., Toouli, J., Herrera, M. F., Kulseng, B., Zulewski, H., Brancatisano, R., … Billington, C. J. (2013). Vagal blocking improves glycemic control and elevated blood pressure in obese subjects with type 2 diabetes mellitus. *Journal of Obesity, 2013*, 245683. https://doi.org/10.1155/2013/245683

Siavash, M., Tabbakhian, M., Sabzghabaee, A. M., & Razavi, N. (2017). Severity of gastrointestinal side effects of metformin tablet compared to metformin capsule in type 2 diabetes mellitus patients. *Journal of Research in Pharmacy Practice, 6*, 73–76. https://doi.org/10.4103/jrpp.jrpp_17_2

Spiller, H. A., & Sawyer, T. S. (2006). Toxicology of oral antidiabetic medications. *American Journal of Health System Pharmacy, 63*, 929–938. https://doi.org/10.2146/ajhp050500

Stauss, H. M., Stangl, H., Clark, K. C., Kwitek, A. E., & Lira, V. A. (2018). Cervical vagal nerve stimulation impairs glucose tolerance and suppresses insulin release in conscious rats. *Physiol Rep, 6*, e13953. https://doi.org/10.14814/phy2.13953

Stumvoll, M., Goldstein, B. J., & van Haften, T. W. (2005). Type 2 diabetes: Principles of pathogenesis and therapy. *Lancet, 365*, 1333–1346. https://doi.org/10.1016/S0140-6736(05)61032-X

Tanaka, T., Nabatame, H., & Tanifuji, Y. (2005). Insulin secretion and glucose utilization are impaired under general anesthesia with sevoflurane as well as isoflurane in a concentration-independent manner. *Journal of Anesthesia, 19*, 277–281. https://doi.org/10.1007/s00504-005-0341-1

Teff, T. K. (2008). Visceral nerves: Vagal and sympathetic innervation. *Journal of Parenteral and Enteral Nutrition, 32*, 569–571. https://doi.org/10.1177/0148607108321705

Tweden, K. S., Anvari, M., Bierk, M. D., Billington, C. J., Camilleri, M., Honda, C. N., … Freston, J. W. (2006). Vagal blocking for obesity control (VBLOC): Concordance of effects of very high frequency blocking current at the neural and organ levels using two preclinical models. *Gastroenterology, 130*.

Tweden, K. S., Sarr, M. G., Camilleri, M., Kendrick, M. L., Moody, F. G., Bierk, M. D., … Anvari, M. (2006). Vagal blocking for obesity control (VBLOC): Studies of pancreatic and gastric function and safety in a porcine model. *Surgery for Obesity and Related Diseases, 2*.

Vetere, A., Choudhary, A., Burns, S. M., & Wagner, B. K. (2014). Targeting the pancreatic beta-cell to treat diabetes. *Nature Reviews Drug Discovery, 13*, 278–289.

Waise, T. M. Z., Dranse, H. J., & Lam, T. K. T. (2018). The metabolic role of vagal afferent innervation. *Nature Reviews Gastroenterology & Hepatology, 15*, 625–636. https://doi.org/10.1038/s41575-018-0062-1

Walz, L., Pettersson, B., Rosenqvist, U., Deleskog, A., Journath, G., & Wandell, P. (2014). Impact of symptomatic hypoglycemia on medication adherence, patient satisfaction with treatment, and glycemic control in patients with type 2 diabetes. *Patient Preference and Adherence, 8*, 593–601. https://doi.org/10.2147/PPA.S58781

Yin, J., Ji, F., Gharibani, P., & Chen, J. D. (2019). Vagal nerve stimulation for glycemic control in a rodent model of type 2 diabetes. *Obesity Surgery, 29*, 2869–2877. https://doi.org/10.1007/s11695-019-03901-9

Zheng, Y., Ley, S. H., & Hu, F. B. (2018). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology, 14*, 88–98. https://doi.org/10.1038/nrendo.2017.151

How to cite this article: Payne SC, Ward G, MacIsaac RJ, Hyakumura T, Fallon JB, Villalobos J. Differential effects of vagus nerve stimulation strategies on glycemia and pancreatic secretions. *Physiol Rep*. 2020;8:e14479. https://doi.org/10.14814/phy2.14479
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Title:
Differential effects of vagus nerve stimulation strategies on glycemia and pancreatic secretions

Date:
2020-06-01

Citation:
Payne, S. C., Ward, G., MacIsaac, R. J., Hyakumura, T., Fallon, J. B. & Villalobos, J. (2020). Differential effects of vagus nerve stimulation strategies on glycemia and pancreatic secretions. PHYSIOLOGICAL REPORTS, 8 (11), https://doi.org/10.14814/phy2.14479.

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