Assessment of the potential for autonomic neuromodulation to reduce perioperative complications and pain: a systematic review and meta-analysis.

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Abstract

Background: Autonomic dysfunction promotes organ injury after major surgery through numerous pathological mechanisms. Vagal withdrawal is a key feature of autonomic dysfunction, and may also increase severity of pain. We systematically evaluated studies that examined whether vagal neuromodulation may have the potential to reduce perioperative complications and pain.

Methods: Two independent reviewers searched PubMed, EMBASE and the Cochrane Register of Controlled Clinical Trials for studies of vagal neuromodulation in humans. Risk of bias was assessed; I^2 index quantified heterogeneity. Primary outcomes were organ dysfunction (assessed by measures of cognition, cardiovascular function and inflammation) and pain. Secondary outcomes were autonomic measures. Standardised mean difference (SMD) using the inverse variance random-effects model with 95%CI summarised effect sizes for continuous outcomes.

Results: From 1258 records, 166 full-text articles were retrieved, of which 31 studies involving patients (n=721) or volunteers (n=679) met the inclusion criteria. Six studies involved interventional cardiology or surgical patients. Indirect stimulation modalities (auricular (n=23) or cervical transcutaneous (n=5)) were most common. Vagal neuromodulation reduced pain (n=10 studies; SMD:2.29 [1.08-3.50]; p=0.0002; I^2=97%) and inflammation (n=6 studies; SMD:1.31 [0.45-2.18]; p=0.003; I^2=91%), and improved cognition (n=11 studies; SMD:1.74 [0.96-2.52]; p<0.0001; I^2=94%) and cardiovascular function (n=6 studies; SMD:3.28 [1.96-4.59]; p<0.00001; I^2=96%). 5/6 studies demonstrated autonomic changes after vagal neuromodulation, by measuring heart rate variability and/or muscle sympathetic nerve activity.

Conclusions: Indirect vagal neuromodulation improves physiological measures associated with limiting organ dysfunction, although studies are of low quality, are

susceptible to bias and lack specific focus on perioperative patients.

Keywords: Neuromodulation; Parasympathetic dysfunction; Organ injury; Perioperative care; Surgery; Critical care

Introduction

Poor exercise capacity in patients undergoing major noncardiac surgery is associated with a higher rate of complications.[1] Cardiac vagal activity is a direct determinant of exercise capacity.[2] Patients at higher risk of complications after noncardiac surgery are characterised by reduced vagal tone[3, 4] (the major determinant of resting heart rate) [5] and impaired arterial baroreflex control.[6, 7] Major noncardiac surgery and/or ensuing critical illness lead to further reductions in vagal activity[4, 8] and, therefore, autonomic imbalance.

Independent of suboptimal exercise capacity, laboratory and clinical translational data also show that preventing the loss of, or restoring, vagal activity limits systemic inflammation,[9] myocardial injury,[10, 11] atrial and ventricular cardiac arrythmias,[12, 13] gastrointestinal complications[14, 15] and lung injury.[16, 17] Moreover, either direct vagal nerve stimulation,[18] or stimulation of vagal afferents,[19] reduces pain in experimental models. Since perioperative morbidity typically involves several organs,[20] the restoration (or prevention of the loss of) vagal activity represents a viable therapeutic target to improve clinical outcomes after major surgery. Device-based vagal neuromodulation has gained traction as a therapeutic option in heart failure, where autonomic dysfunction is a key pathological feature.[21] However, direct electrical stimulation of the vagus nerve is costly, invasive, imprecise and impractical to meet the likely number of high-risk surgical patients who may benefit from re-dressing autonomic imbalance. Alternative, cheaper non-invasive methods of indirect (transcutaneous cervical or auricular) vagal neuromodulation have also been developed.[22]

We hypothesize that autonomic vagal neuromodulation may improve pain and preserve function in organs that frequently sustain injury after noncardiac surgery. To

devise a perioperative therapeutic strategy, it is essential to understand whether similar neuromodulation approaches and/or settings may be beneficial to preserve function of various organs subjected to experimental or clinical stressors. Therefore, we systematically reviewed the literature in order to assess whether autonomic neuromodulation may play a role in attenuating end-organ dysfunction relevant to perioperative medicine and pain.

Methods

Protocol and registration

We registered this systematic review prospectively with PROSPERO: CRD42020216516. The review was performed in adherence to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist.[23] Ethical approval was not required.

Inclusion criteria

Original research articles were considered if they met the following inclusion criteria: adults ≥18 years; randomized controlled trials, or clinical trials in which each individual acted as their own control and the timing of the intervention was not randomized; neuromodulation intervention targeting the vagus nerve; primary endpoint of organ-specific function including (but not limited to) biomarkers of inflammation, cardiovascular, gastrointestinal, and/or cognitive function or pain (as defined by experimental pain thresholds and/or pain symptoms).

Exclusion criteria

We excluded studies where: ex-vivo readouts and/or non-human subjects were used; interventional techniques were used that did not specifically target vagus nerve activity (e.g. acupuncture); stimulation parameters for both intervention and control groups were insufficiently described; all outcome measurements were recorded more than 7 days after the intervention (to ascertain whether any effects occurred within the acute postoperative timeframe).

Information sources and search

We searched PubMed, EMBASE and the Cochrane Central Register of Controlled Clinical Trials (from the inception of each database until 31st October 2020) for relevant publications reporting data on vagal neuromodulation with the assessment of end-organ injury relevant to perioperative medicine and pain. Only randomized controlled trials (or clinical trials in which each individual acted as their own control and the timing of the intervention was not randomized) involving human participants were selected. Results were combined by the Boolean operator "AND" or "OR" with search terms. The PubMed search strategy is provided below as an example:

1. "vagus nerve stimulation" AND "inflammation"
2. "vagus nerve stimulation" AND "pain"
3. "vagus nerve stimulation" AND ("myocardial ischemia" OR "myocardial infarction" OR "atrial fibrillation")
4. "vagus nerve stimulation" AND "gastrointestinal function"
5. "vagus nerve stimulation" AND "cognition"

The searches were conducted independently by two authors (A.B.U.P and V.W), and reviewed by another co-author (G.L.A) for consistency. Differences between the reviewers were addressed through re-examination of the original sources until consensus was reached. No search filters or language restrictions were applied. We extracted records to EndNote (Thomson, Reuters, Philadelphia, PA, USA) to sort and remove duplicates.

Study selection

Studies were selected for inclusion by two authors (A.B.U.P., V.W.) acting independently. After merging the search results and removing duplicates, we screened the titles and abstracts. Full articles that met the inclusion criteria were retrieved.

References of selected articles and published systematic reviews were also searched to identify any further relevant articles meeting the inclusion criteria. The authors of relevant papers were contacted for missing information where possible. When there was uncertainty regarding eligibility, a third reviewer was consulted (G.L.A.).

Data collection process and data items

Data extracted for comparison from the included studies were tabulated by two independent reviewers (A.B.U.P., V.W.) detailing: primary author, year of publication, study design, number of participating patients or volunteers, type of the intervention and control, stimulation parameters, outcomes, and time of intervention and outcome measurement. Means with standard deviation were extracted for continuous outcomes and numbers of events were extracted for dichotomous outcomes.

Primary outcome

1. Study-specific readouts of organ function relevant to the perioperative period (cognitive function, inflammation, cardiovascular, lung and gastrointestinal) and pain.

Secondary outcomes

1. Estimates of vagal activity.
2. Adverse effects of autonomic neuromodulation technique used.

Risk of bias.

Risk of bias was assessed using the Cochrane Risk of Bias Tool for randomized controlled trials.[24] Risk of bias was assessed under the following six domains:

selection, performance, detection, attrition, reporting, and other. Two review authors (A.B.U.P., V.W.) independently assessed the risk of bias. When a consensus could not be reached through discussion, a third reviewer was consulted (G.L.A.).

Statistical analysis

The meta-analysis was conducted using Review Manager software (RevMan; Computer program; Version 5.3; Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Data entry was carried out by two investigators (A.B.U.P. and V.W.) acting independently. For primary outcomes, an inverse variance/random effects model, with the standardised mean difference (SMD) was used for analyses as clinical and methodological heterogeneity was expected. SMD reflects the size of the intervention effect of each study and allows for group comparisons independently of specific outcome measures. A value of zero indicates no effect.[25] We defined the pre-specified threshold for statistical significance as $P<0.05$. Between-study heterogeneity was assessed using the I^2 statistic test using $P<0.1$ as the pre-defined threshold for statistical significance. We defined significant heterogeneity by $I^2 \geq 50\%$. Results are presented as SMD with 95% confidence interval (CI), associated p-values, and forest plots. Potential publication bias was assessed with visual assessment of funnel plots for each meta-analysis outcome.

Results

Study selection

We initially identified 1,258 publications; following removal of duplicates; 951 publications were screened with 166 publications eligible for full text review, leaving 31 publications for analysis (Figure 1). The majority (28/31) of studies used either transcutaneous auricular (n=23) or transcutaneous cervical (n=5) vagal nerve stimulation (VNS) (Figure 2A). One study involved transvenous VNS at spinal level C5–C7, where the internal jugular vein runs adjacent to the vagus nerve.[26] None of the included studies appear to have been supported by the VNS device manufacturers. Six studies were conducted in patients undergoing surgical or interventional cardiology procedures. For VNS stimulation settings, pulse width differed between study types (Figure 2B) but pulse frequency was similar across studies (Figure 2C).

Risk of bias within and across studies

The risk of selection bias was low to moderate, with details on random sequence generation and allocation concealment lacking in numerous trials (Figures 2D-E). The risk of performance and detection bias was moderate to high, as several trials did not report if and how study participants and investigators were blinded to the intervention and outcome measurements. The risk of reporting and other types of bias was low for most studies. The majority of studies (26/31 (84%)) used sham controls.

Primary outcome

Pain

Ten studies explored the impact of vagal neuromodulation (Table 1) on pain intensity,[27-31] subjective pain scores [32] and/or evoked potentials and pain thresholds.[31,

[33-36] Sites of stimulation were either the tragus or cymba conchae for studies using transauricular VNS (Figure 2A). The majority (7/10) reported that VNS increased experimental pain thresholds (healthy volunteers) or improved pain symptoms (patients) (SMD:2.29 [1.08-3.50]; p=0.0002; I^2=97%; Figure 3A). Two studies found that transauricular VNS at the cymba conchae reduced pain induced by heat and pressure.[28, 36] A high level of publication bias was likely, as indicated by asymmetrical funnel plot shape for the primary outcome measures (Figure 3B).

Cognition

Eleven studies reported that cognitive function was improved by mostly transauricular VNS (sites of stimulation were either the tragus or cymba conchae) [37-47] (Table 1) compared with sham stimulation (SMD:1.74 [0.96-2.52]; p<0.0001; I^2=94%; Figure 3C). Studies in volunteers were not age-matched and did not use comparable models. The majority of studies used similar stimulation parameters for transauricular VNS. A high level of publication bias was likely, as indicated by asymmetrical funnel plot shape for the primary outcome measures (Figure 3D).

Systemic Inflammation

5/6 studies using similar stimulation parameters reported a reduction in circulating cytokine levels after variable periods of VNS (Table 2)[48-52] compared with sham stimulation (SMD:1.31 [0.45-2.18]; p=0.003; I^2=91%; Figure 3E). In a randomized double-blind sham-controlled trial of experimental human endotoxemia, transvenous VNS failed to reduce symptoms, cytokine levels or measures of the innate immune response.[26] A low level of publication bias was likely, as indicated by symmetrical funnel plot shape for the primary outcome measures (Figure 3F).

Cardiovascular

Six studies reported that autonomic neuromodulation by VNS reduces cardiovascular morbidity and/or mortality (Table 3), compared with the control group (SMD:3.28 [1.96-4.59]; $p<0.00001$; $I^2=96\%$; Figure 3G). Three studies reported that VNS improved echocardiographic indices and/or biomarkers for severity of heart failure.[52-54] Two studies found that VNS suppressed atrial fibrillation in individuals undergoing cardiac surgery and those with paroxysmal AF.[49, 50] Auricular VNS also reduced levels of biomarkers for myocardial ischaemia, improved left ventricular contractility and reduced reperfusion-related arrhythmias in two studies conducted in patients undergoing percutaneous coronary intervention for acute myocardial infarction [52] and bypass graft surgery for coronary artery disease.[54] A high level of publication bias was likely, as indicated by asymmetrical funnel plot shape for the primary outcome measures (Figure 3H).

Other organ systems

3/4 (75%) studies reported that VNS improved gastrointestinal motility [34, 48, 55] compared with sham stimulation (Supplementary Table 1). A single study found that transauricular VNS reduced the incidence of postoperative pneumonia and pro-inflammatory cytokine levels.[51]

Secondary outcome: estimates of autonomic modulation

Only 6/31 studies determined whether VNS altered autonomic function, of which 5 reported higher vagal activity and/or reductions of sympathetic activity, as reflected

by heart rate variability analysis (Supplementary Table 2), muscle sympathetic nerve activity and inhibition of noradrenaline release.[54]

Adverse effects of vagal neuromodulation techniques

One study reported more burning/stinging sensation with VNS than with sham stimulation.[38] Common adverse effects were otherwise not reported although there were no safety concerns noted with any intervention. Only 7/31 studies explicitly reported on safety, tolerability and adverse effects.

Discussion

Our prospectively registered systematic review suggests that autonomic neuromodulation aimed at increasing or preserving vagal activity in non-surgical settings has the potential to reduce organ dysfunction relevant to perioperative medicine and pain. The majority of studies used transauricular VNS. Few studies met the minimum reporting standards for transcutaneous VNS-based research, as recommended by an international consensus-based review,[22] which include detailing the precise site for auricular stimulation. We noted that VNS applied 24 hours before surgical or interventional cardiology procedures reduced inflammation and/or organ injury, although only one study was conducted in noncardiac surgical patients. Although only ~20% studies directly examined the autonomic effects of stimulation, these findings suggest that transauricular VNS offers therapeutic potential for common complications that occur during the perioperative period.

We specifically looked at proof-of-concept studies in patients and human volunteers that inform whether the intervention may be useful in the perioperative setting. Pain, cognitive dysfunction (in part caused by injury to brain tissue) and dysfunction of other organs cluster together following major surgery.[56] Many of the volunteer studies involved individuals who were administered painful stimuli [27, 28, 31, 35, 36] or found to have underlying subclinical neuropsychiatric states (eg. anxiety, depression, confusion, post-traumatic stress and memory disorders)[37, 38, 43, 44] before VNS was administered. Whilst these subjects have been selected because they are deemed to be healthy (and often young) without any morbidity, they still experience pain and cognitive challenges prior to surgery. Indeed, preoperative anxiety and pain are clinically relevant modifiers of surgical stress and

inflammation.[57] Proof-of-concept data from the volunteer studies are useful for the assessment of the potential for vagal neuromodulation to reduce organ dysfunction relevant to perioperative medicine and pain. By analogy, a novel analgesic would not be introduced into the perioperative setting without similar proof-of-concept studies in healthy volunteers.

Similarly, interventions aimed at preserving cognition would require proof-of-concept studies in human volunteers before moving into the perioperative arena. Several routine perioperative interventions can affect cognition in healthy subjects. For example, an experimental study conducted in young healthy volunteers undergoing brachial plexus blockade assessed cognitive performance. Change in cognition before and after brachial plexus blockade were assessed using a left/right hand task, involving motor imagery processes and perception illusions pertaining to hand posture. Participants performed less quickly and accurately on the task during regional anaesthesia. These findings suggested that brachial plexus anaesthesia disrupts cognition relevant to the perioperative period.[58] General anaesthetic agents [59] and opioids also reduce memory and/or cognitive performance, as assessed by performing executive function tasks.[60] Therefore, demonstration of efficacy of vagal neuromodulation in healthy younger subjects is relevant to perioperative care, even though they are not likely to be at risk of serious cognitive impairment. However, our review of healthy volunteer studies in this context should be treated with caution, as these studies measured surrogate measures of cognitive dysfunction.

Non-electrical, non-pharmacological techniques such as sham feeding with chewing gum also preserve and/or augment vagal activity and reduce gastrointestinal dysfunction,[61, 62] although many operations preclude this approach.

Nevertheless, these physiological interventional trials support the paradigm that vagal tone may be modulated to improve gastrointestinal motility disorders and reduce pain. Although very few studies have attempted to directly modulate autonomic function in the perioperative setting, acupuncture has demonstrated a potential perioperative role.[63, 64, 65] Acupuncture reduces the consumption of anaesthetics and analgesics.[63] Perioperative acupuncture reduced perioperative opioid consumption, in concert with a 30-50% reduction in plasma cortisol and epinephrine within 24h of surgery.[66] Transcutaneous electrical nerve stimulation also reduces postoperative incisional site pain,[67, 68] targeting the ilioinguinal, iliohypogastric and/or genitofemoral nerves. In the context of our findings, auricular acupuncture also appears to be effective for the treatment of preoperative anxiety. Aside from the need for advanced and specific skills, the exact mechanism of action, neurophysiologic target and efficacy of acupuncture remain to be established. The neurophysiology underlying acupuncture is highly relevant to VNS. Acupuncture reduces splenic and serum inflammatory responses in experimentally induced acute inflammation.[69] Vagotomy and splenic neurectomy reverses these anti-inflammatory effects, suggesting that acupuncture stimulation may confer its therapeutic benefits via vagal neuromodulation of inflammatory responses observed in the spleen, and potentially other organs.[69] A meta-analysis of 17 randomized controlled trials has demonstrated that 75% of localized auricular acupuncture points, targeted during pain therapy, are found in regions predominantly innervated by the auricular branch of the vagus nerve (ABVN).[70] These clinical findings are consistent with the paradigm that stimulation of the ABVN may be a key analgesic mechanism of auricular acupuncture.[70]

Patients who develop complications after noncardiac surgery rarely experience single organ dysfunction.[20] Even apparently relatively minor

complications reduce life expectancy, a finding that has been repeatedly reported across different healthcare systems for the last three decades.[71, 72, 73] Mechanistically, the clustering of complications is plausibly driven by the failure of inter-organ crosstalk, which is required for effective resolution of inflammation and minimising organ injury. The clustering of pain with other postoperative complications is common and may directly promote and/or prolong other complications.[74] The majority of VNS studies assessing pain and pain threshold outcomes involved the administration of painful stimuli, bearing clear relevance to the surgical period. However, an increase in a pain threshold (while indicative of an analgesic effect) does not necessarily translate to organ protection, and may increase the risk of organ dysfunction through numerous mechanisms.

Translational and clinical studies have demonstrated that maintained and/or augmented efferent vagal activity reduces the pathophysiological effects of renal,[75] neurological [76] and ventilator-induced lung injury [17] and provides cardioprotection.[10, 11] Aside from direct organ innervation, experimental data also show that augmenting vagal activity limits systemic inflammation through innate and adaptive immune mechanisms. VNS reduces haemorrhage in experimental surgical tissue injury by increasing coagulation factor activity.[77]

A substantial number of noncardiac surgical patients at higher risk of complications demonstrate vagal autonomic dysfunction before surgery, which is strongly linked to reduced exercise capacity.[2] Mechanistically, inhibition of vagal neurons in the dorsal vagal motor nucleus reduce exercise capacity by 80% in rats.[5] Conversely, activating the same neurons optogenetically enhances exercise capacity to the same degree as treadmill training.[5] Thus, vagal dysfunction is likely to be a key feature of high-risk, deconditioned surgical patients. These observations are supported

by the high prevalence of preoperative baroreflex dysfunction in surgical patients, which is associated with impaired cardiac function, and an inability to respond to goal directed therapy.[7] After surgery, prolonged bed rest impairs baroreflex sensitivity,[78, 79] which is linked to increased risk of postoperative cardiac and infectious complications [80] as well as mortality.[81]

Postoperative delirium and cognitive dysfunction (POCD) occur commonly after noncardiac surgery and are associated with prolonged hospitalisation and higher mortality.[82] Neuroinflammation contributes to POCD.[83] Transcutaneous cervical VNS reduced inflammation generated by microglia in a murine model of Alzheimer's disease.[84] A similar effect on hippocampal inflammation has been demonstrated in a model of exploratory laparotomy in aged mice.[85] The positive impact of VNS in numerous cognitive studies in humans suggests that this approach may also have perioperative utility in reducing POCD.

The drawbacks of the volunteer studies that assess pain and cognition are that they do not involve patients with organ dysfunction. However, as considered before, these studies do provide proof-of-concept data. Pain and cognition are domains associated with worse postoperative outcomes; about 12% of individuals with no evidence of preoperative cognitive dysfunction will develop symptoms of POCD following anaesthesia and noncardiac surgery,[86] and postoperative pain is poorly controlled in many apparently previously well patients.[87] Our review is also limited by studies that are not age-matched, do not use comparable models and demonstrate significant heterogeneity. The studies also vary widely in terms of the timepoints at which VNS was applied, with variable outcomes assessed. There is a lack of data on either optimal and/or dose-response.[88] One systematic investigation of three combinations of different pulse width and frequency demonstrated that a pulse width

of 500μs and frequency of 10Hz produced the greatest reduction in heart rate.[89] Whilst no eligible non-English full-text articles were found, the exclusion of case reports and observational cohort and case-control studies are also potential limitations. Although funnel plot analyses provided evidence of publication bias, inclusion of less than 10 studies in individual funnel plots renders real asymmetry difficult to distinguish from chance.[90]

Lack of blinding is a further potential limitation, although sham stimulation served as an effective control for three reasons. First, in most studies, investigators measuring outcomes were masked to treatment allocations. Second, many studies were designed so that the stimulation settings were not observable or detectable to participants; investigators increased the current intensity until it was detectable, before reducing the intensity to just below this tactile threshold (for active stimulation) or off (for sham stimulation). Placebo interventions are effective, particularly within the context of pain.[91] Therefore, for the included studies in which the stimulation settings were neither observable nor detectable to subjects, any difference detected between sham and stimulation intervention groups is likely attributable to VNS. However, a minority of studies were designed such that subjects could distinguish between sham and active stimulation. Third, ~50% of the trials were crossover studies, in which each subject acted as their own control for both sham and stimulation interventions.

The cumulative results report an improvement of ~2-3 SMD in treatment effect, supporting the use of VNS in perioperative patients. However, the majority of studies are small (<30 subjects), and many studies report inadequate or poor evidence of allocation concealment and/or blinding of participants, investigators

and outcome assessments. This increases susceptibility to high levels of bias, a point emphasized by the asymmetrical funnel plots. Therefore, the poor quality of studies necessitates larger studies with better design and control, before it can be concluded that VNS should universally be used perioperatively.

In summary, current studies are of low quality, susceptible to publication bias and utilise surrogate measures with an implied link to actual perioperative organ dysfunction. However, several proof-of-concept studies in humans suggest that autonomic neuromodulation strategies aimed at maintaining or augmenting vagal activity may be utilised to reduce organ dysfunction and pain during the perioperative period but definitive studies are lacking.

Authors' Contributions: A.B.U.P developed the protocol and performed the systematic search, data extraction, analysis and write up for this review. V.W performed the systematic search, data extraction and analysis. G.L.A and A.V.G supervised this review from development of protocol through to write up.

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Conflicts of Interest: GLA: Editor, British Journal of Anaesthesia; consultancy work for GlaxoSmithKline, unrelated to this work. AVG is a founder and Chief Scientific Officer for Afferent Medical Solutions. The other authors declare no competing financial interests.

Figure legends.

Figure 1. PRISMA flow diagram of literature search results. Thirty-one randomized controlled trials were included for meta-analysis. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

Figure 2. Study design and quality.

A. Modes of stimulation, as indicated by red shading. Transcutaneous auricular vagus nerve stimulation is highlighted within the ear (which the majority of studies utilise). B. Pulse width settings. Data are presented as mean (SD) and analysed using one-way ANOVA. P values refer to post-hoc Tukey Kramer tests conducted to determine between factor differences. Pulse width (μs) settings were different between cardiovascular and cognition studies, cognition and inflammation studies, and inflammation and pain studies. C. Pulse frequency settings. Pulse frequency (Hz) settings were similar between all cardiovascular, cognition, inflammation and pain studies ($p>0.05$).

D. Cumulative risks of bias (by subtype): assessment of each risk of bias item shown as percentages for all included studies. E. Cumulative risks of bias (by study): assessment of each risk of bias item for all included studies. Green – low risk; yellow – insufficient data; red – high risk.

Figure 3. Effects of VNS on organ function and pain.

Pain: A. Forest plot analysis showed VNS reduced pain compared with the control group (SMD:2.29 [1.08-3.50]; $p=0.0002$; $I^2=97\%$). B. Funnel plot analysis showed asymmetrical shape, suggestive of a high level of publication bias.

Cognition: C. Forest plot analysis showed VNS improved cognitive function compared with the control group (SMD:1.74 [0.96-2.52]; p<0.0001; I^2=94%). D. Funnel plot analysis showed asymmetrical shape, suggestive of a high level of publication bias.

Inflammation: E. Forest plot analysis showed VNS reduced inflammatory markers compared with the control group (SMD:1.31 [0.45-2.18]; p=0.003; I^2=91%). F. Funnel plot analysis showed symmetrical shape, suggestive of a low level of publication bias.

Cardiovascular: G. Forest plot analysis showed VNS improved cardiovascular function compared with the control group (SMD:3.28 [1.96-4.59]; p<0.00001; I^2=96%). H. Funnel plot analysis showed asymmetrical shape, suggestive of a high level of publication bias.

CI, confidence interval; SD, standard deviation; VNS, vagus nerve stimulation. The difference in mean values is attributable to heterogeneity of units.

References

1 Wijeysundera DN, Pearse RM, Shulman MA, et al. Assessment of functional capacity before major non-cardiac surgery: an international, prospective cohort study. *Lancet* 2018; **391**: 2631-40
2 Gourine AV, Ackland GL. Cardiac Vagus and Exercise. *Physiology (Bethesda)* 2019; **34**: 71-80
3 Abbott TEF, Pearse RM, Cuthbertson BH, Wijeysundera DN, Ackland GL, investigators Ms. Cardiac vagal dysfunction and myocardial injury after non-cardiac surgery: a planned secondary analysis of the measurement of Exercise Tolerance before surgery study. *Br J Anaesth* 2019; **122**: 188-97
4 May SM, Reyes A, Martir G, et al. Acquired loss of cardiac vagal activity is associated with myocardial injury in patients undergoing noncardiac surgery: prospective observational mechanistic cohort study. *Br J Anaesth* 2019; **123**: 758-67
5 Machhada A, Trapp S, Marina N, et al. Vagal determinants of exercise capacity. *Nat Commun* 2017; **8**: 15097
6 Toner A, Jenkins N, Ackland GL, Investigators P-OS. Baroreflex impairment and morbidity after major surgery. *British journal of anaesthesia* 2016; **117**: 324-31
7 Ackland GL, Whittle J, Toner A, et al. Molecular Mechanisms Linking Autonomic Dysfunction and Impaired Cardiac Contractility in Critical Illness. *Crit Care Med* 2016; **44**: e614-24
8 Ackland GL, Iqbal S, Paredes LG, et al. Individualised oxygen delivery targeted haemodynamic therapy in high-risk surgical patients: a multicentre, randomised, double-blind, controlled, mechanistic trial. *Lancet Respir Med* 2015; **3**: 33-41
9 Chavan SS, Pavlov VA, Tracey KJ. Mechanisms and Therapeutic Relevance of Neuro-immune Communication. *Immunity* 2017; **46**: 927-42
10 Machhada A, Hosford PS, Dyson A, Ackland GL, Mastitskaya S, Gourine AV. Optogenetic Stimulation of Vagal Efferent Activity Preserves Left Ventricular Function in Experimental Heart Failure. *JACC Basic Transl Sci* 2020; **5**: 799-810
11 Mastitskaya S, Marina N, Gourine A, et al. Cardioprotection evoked by remote ischaemic preconditioning is critically dependent on the activity of vagal pre-ganglionic neurones. *Cardiovasc Res* 2012; **95**: 487-94
12 Machhada A, Gourine AV, Ackland GL. Vagal Modulation of Atrial Fibrillation. *J Am Coll Cardiol* 2015; **66**: 977-8
13 Machhada A, Ang R, Ackland GL, et al. Control of ventricular excitability by neurons of the dorsal motor nucleus of the vagus nerve. *Heart Rhythm* 2015; **12**: 2285-93
14 Lubbers T, Kox M, de Haan JJ, et al. Continuous administration of enteral lipid- and protein-rich nutrition limits inflammation in a human endotoxemia model. *Crit Care Med* 2013; **41**: 1258-65
15 Lubbers T, Buurman W, Luyer M. Controlling postoperative ileus by vagal activation. *World journal of gastroenterology : WJG* 2010; **16**: 1683-7
16 Levy G, Fishman JE, Xu DZ, et al. Vagal nerve stimulation modulates gut injury and lung permeability in trauma-hemorrhagic shock. *J Trauma Acute Care Surg* 2012; **73**: 338-42; discussion 42
17 dos Santos CC, Shan Y, Akram A, Slutsky AS, Haitsma JJ. Neuroimmune regulation of ventilator-induced lung injury. *Am J Respir Crit Care Med* 2011; **183**: 471-82
18 Randich A, Aicher SA. Medullary substrates mediating antinociception produced by electrical stimulation of the vagus. *Brain Res* 1988; **445**: 68-76
19 Randich A, Ren K, Gebhart GF. Electrical stimulation of cervical vagal afferents. II. Central relays for behavioral antinociception and arterial blood pressure decreases. *J Neurophysiol* 1990; **64**: 1115-24

20 Ackland GL, Abbott TEF, Jones TF, et al. Early elevation in plasma high-sensitivity troponin T and morbidity after elective noncardiac surgery: prospective multicentre observational cohort study. *Br J Anaesth* 2020; **124**: 535-43
21 Schwartz PJ, La Rovere MT, De Ferrari GM, Mann DL. Autonomic modulation for the management of patients with chronic heart failure. *Circ Heart Fail* 2015; **8**: 619-28
22 Farmer AD, Strzelczyk A, Finisguerra A, et al. International Consensus Based Review and Recommendations for Minimum Reporting Standards in Research on Transcutaneous Vagus Nerve Stimulation (Version 2020). *Front Hum Neurosci* 2020; **14**: 568051
23 Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; **6**: e1000097
24 Higgins JPT, Sterne JAC, Savović J. A revised tool for assessing risk of bias in randomized trials. *Cochrane methods* 2016;
https://www.riskofbias.info/welcome/rob-2-0-tool:
25 Higgins J, Green S. *Cochrane handbook for systematic reviews of interventions.* www.handbook.cochrane.org, 2011
26 Kox M, van Eijk LT, Verhaak T, et al. Transvenous vagus nerve stimulation does not modulate the innate immune response during experimental human endotoxemia: a randomized controlled study. *Arthritis Res Ther* 2015; **17**: 150
27 Lerman I, Davis B, Huang M, et al. Noninvasive vagus nerve stimulation alters neural response and physiological autonomic tone to noxious thermal challenge. *PLoS One* 2019; **14**: e0201212
28 Janner H, Klausenitz C, Gurtler N, Hahnenkamp K, Usichenko TI. Effects of Electrical Transcutaneous Vagus Nerve Stimulation on the Perceived Intensity of Repetitive Painful Heat Stimuli: A Blinded Placebo- and Sham-Controlled Randomized Crossover Investigation. *Anesth Analg* 2018; **126**: 2085-92
29 Napadow V, Edwards RR, Cahalan CM, et al. Evoked pain analgesia in chronic pelvic pain patients using respiratory-gated auricular vagal afferent nerve stimulation. *Pain Med* 2012; **13**: 777-89
30 Kutlu N, Ozden AV, Alptekin HK, Alptekin JO. The Impact of Auricular Vagus Nerve Stimulation on Pain and Life Quality in Patients with Fibromyalgia Syndrome. *Biomed Res Int* 2020; **2020**: 8656218
31 Alt LK, Wach K, Liebler EJ, Straube A, Ruscheweyh R. A Randomized Sham-Controlled Cross-Over Study on the Short-Term Effect of Non-Invasive Cervical Vagus Nerve Stimulation on Spinal and Supraspinal Nociception in Healthy Subjects. *Headache* 2020; **60**: 1616-31
32 Tassorelli C, Grazzi L, de Tommaso M, et al. Noninvasive vagus nerve stimulation as acute therapy for migraine: The randomized PRESTO study. *Neurology* 2018; **91**: e364-e73
33 De Icco R, Martinelli D, Bitetto V, et al. Peripheral vagal nerve stimulation modulates the nociceptive withdrawal reflex in healthy subjects: A randomized, cross-over, sham-controlled study. *Cephalalgia* 2018; **38**: 1658-64
34 Frokjaer JB, Bergmann S, Brock C, et al. Modulation of vagal tone enhances gastroduodenal motility and reduces somatic pain sensitivity. *Neurogastroenterol Motil* 2016; **28**: 592-8
35 Laqua R, Leutzow B, Wendt M, Usichenko T. Transcutaneous vagal nerve stimulation may elicit anti- and pro-nociceptive effects under experimentally-induced pain - a crossover placebo-controlled investigation. *Auton Neurosci* 2014; **185**: 120-2
36 Busch V, Zeman F, Heckel A, Menne F, Ellrich J, Eichhammer P. The effect of transcutaneous vagus nerve stimulation on pain perception--an experimental study. *Brain Stimul* 2013; **6**: 202-9

37 Bretherton B, Atkinson L, Murray A, Clancy J, Deuchars S, Deuchars J. Effects of transcutaneous vagus nerve stimulation in individuals aged 55 years or above: potential benefits of daily stimulation. *Aging (Albany NY)* 2019; **11**: 4836-57
38 Burger AM, Van der Does W, Thayer JF, Brosschot JF, Verkuil B. Transcutaneous vagus nerve stimulation reduces spontaneous but not induced negative thought intrusions in high worriers. *Biol Psychol* 2019; **142**: 80-9
39 Fischer R, Ventura-Bort C, Hamm A, Weymar M. Transcutaneous vagus nerve stimulation (tVNS) enhances conflict-triggered adjustment of cognitive control. *Cogn Affect Behav Neurosci* 2018; **18**: 680-93
40 Colzato LS, Ritter SM, Steenbergen L. Transcutaneous vagus nerve stimulation (tVNS) enhances divergent thinking. *Neuropsychologia* 2018; **111**: 72-6
41 Colzato LS, Wolters G, Peifer C. Transcutaneous vagus nerve stimulation (tVNS) modulates flow experience. *Exp Brain Res* 2018; **236**: 253-7
42 Sellaro R, van Leusden JW, Tona KD, Verkuil B, Nieuwenhuis S, Colzato LS. Transcutaneous Vagus Nerve Stimulation Enhances Post-error Slowing. *J Cogn Neurosci* 2015; **27**: 2126-32
43 Jacobs HI, Riphagen JM, Razat CM, Wiese S, Sack AT. Transcutaneous vagus nerve stimulation boosts associative memory in older individuals. *Neurobiol Aging* 2015; **36**: 1860-7
44 Wittbrodt MT, Gurel NZ, Nye JA, et al. Non-invasive vagal nerve stimulation decreases brain activity during trauma scripts. *Brain Stimul* 2020; **13**: 1333-48
45 Borges U, Knops L, Laborde S, Klatt S, Raab M. Transcutaneous Vagus Nerve Stimulation May Enhance Only Specific Aspects of the Core Executive Functions. A Randomized Crossover Trial. *Front Neurosci* 2020; **14**: 523
46 Warren CV, Maraver MJ, de Luca A, Kopp B. The Effect of Transcutaneous Auricular Vagal Nerve Stimulation (taVNS) on P3 Event-Related Potentials during a Bayesian Oddball Task. *Brain Sci* 2020; **10**
47 Steenbergen L, Sellaro R, Stock AK, Verkuil B, Beste C, Colzato LS. Transcutaneous vagus nerve stimulation (tVNS) enhances response selection during action cascading processes. *Eur Neuropsychopharmacol* 2015; **25**: 773-8
48 Stakenborg N, Labeeuw E, Gomez-Pinilla PJ, et al. Preoperative administration of the 5-HT4 receptor agonist prucalopride reduces intestinal inflammation and shortens postoperative ileus via cholinergic enteric neurons. *Gut* 2019; **68**: 1406-16
49 Stavrakis S, Humphrey MB, Scherlag B, et al. Low-Level Vagus Nerve Stimulation Suppresses Post-Operative Atrial Fibrillation and Inflammation: A Randomized Study. *JACC Clin Electrophysiol* 2017; **3**: 929-38
50 Stavrakis S, Humphrey MB, Scherlag BJ, et al. Low-level transcutaneous electrical vagus nerve stimulation suppresses atrial fibrillation. *J Am Coll Cardiol* 2015; **65**: 867-75
51 Salama M, Akan A, Mueller MR. Transcutaneous Stimulation of Auricular Branch of the Vagus Nerve Attenuates the Acute Inflammatory Response After Lung Lobectomy. *World J Surg* 2020; **44**: 3167-74
52 Yu L, Huang B, Po SS, et al. Low-Level Tragus Stimulation for the Treatment of Ischemia and Reperfusion Injury in Patients With ST-Segment Elevation Myocardial Infarction: A Proof-of-Concept Study. *JACC Cardiovasc Interv* 2017; **10**: 1511-20
53 Tran N, Asad Z, Elkholey K, Scherlag BJ, Po SS, Stavrakis S. Autonomic Neuromodulation Acutely Ameliorates Left Ventricular Strain in Humans. *J Cardiovasc Transl Res* 2019; **12**: 221-30
54 Zamotrinsky AV, Kondratiev B, de Jong JW. Vagal neurostimulation in patients with coronary artery disease. *Auton Neurosci* 2001; **88**: 109-16
55 Teckentrup V, Neubert S, Santiago JCP, Hallschmid M, Walter M, Kroemer NB. Non-invasive stimulation of vagal afferents reduces gastric frequency. *Brain Stimul* 2020; **13**: 470-3

56 Grocott MP, Browne JP, Van der Meulen J, et al. The Postoperative Morbidity Survey was validated and used to describe morbidity after major surgery. *J Clin Epidemiol* 2007; **60**: 919-28
57 Manou-Stathopoulou V, Korbonits M, Ackland GL. Redefining the perioperative stress response: a narrative review. *Br J Anaesth* 2019; **123**: 570-83
58 Silva S, Loubinoux I, Olivier M, et al. Impaired visual hand recognition in preoperative patients during brachial plexus anesthesia: importance of peripheral neural input for mental representation of the hand. *Anesthesiology* 2011; **114**: 126-34
59 Goswami U, Babbar S, Tiwari S. Comparative evaluation of the effects of propofol and sevoflurane on cognitive function and memory in patients undergoing laparoscopic cholecystectomy: A randomised prospective study. *Indian J Anaesth* 2015; **59**: 150-5
60 van Steenbergen H, Eikemo M, Leknes S. The role of the opioid system in decision making and cognitive control: A review. *Cogn Affect Behav Neurosci* 2019; **19**: 435-58
61 Karmali S, Jenkins N, Sciusco A, et al. Randomized controlled trial of vagal modulation by sham feeding in elective non-gastrointestinal (orthopaedic) surgery. *Br J Anaesth* 2015; **115**: 727-35
62 van den Heijkant TC, Costes LM, van der Lee DG, et al. Randomized clinical trial of the effect of gum chewing on postoperative ileus and inflammation in colorectal surgery. *Br J Surg* 2015; **102**: 202-11
63 Lu Z, Dong H, Wang Q, Xiong L. Perioperative acupuncture modulation: more than anaesthesia. *Br J Anaesth* 2015; **115**: 183-93
64 Wang SM, Peloquin C, Kain ZN. The use of auricular acupuncture to reduce preoperative anxiety. *Anesth Analg* 2001; **93**: 1178-80, table of contents
65 Wang SM, Kain ZN. Auricular acupuncture: a potential treatment for anxiety. *Anesth Analg* 2001; **92**: 548-53
66 Kotani N, Hashimoto H, Sato Y, et al. Preoperative intradermal acupuncture reduces postoperative pain, nausea and vomiting, analgesic requirement, and sympathoadrenal responses. *Anesthesiology* 2001; **95**: 349-56
67 Smith CM, Guralnick MS, Gelfand MM, Jeans ME. The effects of transcutaneous electrical nerve stimulation on post-cesarean pain. *Pain* 1986; **27**: 181-93
68 Ilfeld BM, Finneran JJ. Cryoneurolysis and Percutaneous Peripheral Nerve Stimulation to Treat Acute Pain. *Anesthesiology* 2020; **133**: 1127-49
69 Lim HD, Kim MH, Lee CY, Namgung U. Anti-Inflammatory Effects of Acupuncture Stimulation via the Vagus Nerve. *PLoS One* 2016; **11**: e0151882
70 Usichenko T, Hacker H, Lotze M. Transcutaneous auricular vagal nerve stimulation (taVNS) might be a mechanism behind the analgesic effects of auricular acupuncture. *Brain Stimul* 2017; **10**: 1042-4
71 Khuri SF, Henderson WG, DePalma RG, Mosca C, Healey NA, Kumbhani DJ. Determinants of long-term survival after major surgery and the adverse effect of postoperative complications. *AnnSurg* 2005; **242**: 326-41
72 Moonesinghe SR, Harris S, Mythen MG, et al. Survival after postoperative morbidity: a longitudinal observational cohort study. *Br J Anaesth* 2014; **113**: 977-84
73 Portuondo JI, Itani KMF, Massarweh NN. Association Between Postoperative Complications and Long-term Survival after Non-cardiac Surgery among Veterans. *Ann Surg* 2021
74 Wikman A, Johar A, Lagergren P. Presence of symptom clusters in surgically treated patients with esophageal cancer: implications for survival. *Cancer* 2014; **120**: 286-93
75 Inoue T, Abe C, Sung SJ, et al. Vagus nerve stimulation mediates protection from kidney ischemia-reperfusion injury through alpha7nAChR+ splenocytes. *J Clin Invest* 2016

76 Cheyuo C, Jacob A, Wu R, Zhou M, Coppa GF, Wang P. The parasympathetic nervous system in the quest for stroke therapeutics. *J Cereb Blood Flow Metab* 2011; **31**: 1187-95
77 Czura CJ, Schultz A, Kaipel M, et al. Vagus nerve stimulation regulates hemostasis in swine. *Shock* 2010; **33**: 608-13
78 Barbic F, Heusser K, Minonzio M, et al. Effects of Prolonged Head-Down Bed Rest on Cardiac and Vascular Baroreceptor Modulation and Orthostatic Tolerance in Healthy Individuals. *Front Physiol* 2019; **10**: 1061
79 Coupe M, Fortrat JO, Larina I, Gauquelin-Koch G, Gharib C, Custaud MA. Cardiovascular deconditioning: From autonomic nervous system to microvascular dysfunctions. *Respir Physiol Neurobiol* 2009; **169 Suppl 1**: S10-2
80 Vinik AI, Erbas T, Casellini CM. Diabetic cardiac autonomic neuropathy, inflammation and cardiovascular disease. *J Diabetes Investig* 2013; **4**: 4-18
81 Schmidt H, Muller-Werdan U, Hoffmann T, et al. Autonomic dysfunction predicts mortality in patients with multiple organ dysfunction syndrome of different age groups. *Crit Care Med* 2005; **33**: 1994-2002
82 Deiner S, Silverstein JH. Postoperative delirium and cognitive dysfunction. *Br J Anaesth* 2009; **103 Suppl 1**: i41-6
83 Nadelson MR, Sanders RD, Avidan MS. Perioperative cognitive trajectory in adults. *Br J Anaesth* 2014; **112**: 440-51
84 Kaczmarczyk R, Tejera D, Simon BJ, Heneka MT. Microglia modulation through external vagus nerve stimulation in a murine model of Alzheimer's disease. *J Neurochem* 2017
85 Cai L, Lu K, Chen X, Huang JY, Zhang BP, Zhang H. Auricular vagus nerve stimulation protects against postoperative cognitive dysfunction by attenuating neuroinflammation and neurodegeneration in aged rats. *Neurosci Lett* 2019; **703**: 104-10
86 Needham MJ, Webb CE, Bryden DC. Postoperative cognitive dysfunction and dementia: what we need to know and do. *Br J Anaesth* 2017; **119**: i115-i25
87 Gan TJ. Poorly controlled postoperative pain: prevalence, consequences, and prevention. *J Pain Res* 2017; **10**: 2287-98
88 Butt MF, Albusoda A, Farmer AD, Aziz Q. The anatomical basis for transcutaneous auricular vagus nerve stimulation. *J Anat* 2020; **236**: 588-611
89 Badran BW, Mithoefer OJ, Summer CE, et al. Short trains of transcutaneous auricular vagus nerve stimulation (taVNS) have parameter-specific effects on heart rate. *Brain Stimul* 2018; **11**: 699-708
90 Sedgwick P, Marston L. How to read a funnel plot in a meta-analysis. *BMJ* 2015; **351**: h4718
91 Wager TD, Atlas LY. The neuroscience of placebo effects: connecting context, learning and health. *Nat Rev Neurosci* 2015; **16**: 403-18