NEURAL REGENERATION RESEARCH
www.nrronline.org

Emerging non-invasive therapeutic approaches targeting hypocholinergic neural systems in Parkinson's disease

Nicolaas I. Bohnen*, Alison J. Yarnall

Cholinergic system associated DOPA-refractory motor and cognitive symptoms – a need for novel therapeutic approaches: Accumulating evidence points to significant motor and non-motor morbidities associated with hypocholinergic deficits in central and peripheral neural systems in Parkinson's disease (PD) (Bohnen et al., 2018, 2022). This so-called "malignant" hypocholinergic disease phenotype is associated with DOPA-refractory dementia and mobility disturbances, such as falls and freezing of gait, and augur novel therapeutic approaches targeting cholinergic systems in PD. Cholinergic pharmacotherapy in PD has been an interest for a long time. However, the development of cholinergic augmentation pharmacotherapy has been hampered by limited clinical efficacy, the presumption that changes in cholinergic activity are homogeneous in the central nervous system (CNS) and peripheral nervous system, tolerance or safety of cholinesterase inhibitor drugs, low CNS penetrance, high rate of peripheral autonomic side-effects and clinical contra-indications. The development of nicotinic or muscarinic receptor modulating drugs appears more promising but is still in the development stage. Given the current unmet need for managing DOPA-refractory cognitive and mobility impairments associated with hypocholinergic neural systems, there is a need for novel and complementary therapeutic and more personalized approaches.

Potential cholinergic targets in PD – non-pharmacological approaches: Cholinergic functions may be modulated either by direct or indirect cholinergic neurostimulation/modulation approaches. Direct cholinergic neurostimulation approaches include invasive neurostimulation of the major cholinergic projections nuclei: the nucleus basalis of Meynert (NBM) or the pedunculopontine-nucleus (PPN) (**Figure 1**). However, preliminary evidence of limited phase Ib clinical trials has shown variable results (Bohnen et al., 2022). These variable results were due to small sample sizes of predominant open-label studies, technical difficulties targeting the PPN anatomic location during surgery, and variation in the selection of programming parameters.

Invasive vagus nerve stimulation (VNS) is another direct cholinergic innervation therapeutic approach. Invasive VNS is in clinical use for the treatment of depression and epilepsy, but not for PD. VNS may stimulate or modulate afferent vagal pathways, and central cholinergic and efferent vagal pathways. There is increasing interest in applying VNS on a non-invasive basis in PD as clinical effects are already seen with intermittent stimulation. More recently, the vagus nerve has attracted much attention as it is an important mediator of the so-called gut-brain axis (GBA) in PD with implications for modulating acetylcholine (ACh)-dependent microbiome and short-chain fatty acid (SCFA) functions that affect the vagus and CNS cholinergic systems.

This perspective will discuss emerging non-invasive therapeutic stimulation approaches targeting CNS and autonomic nervous system hypocholinergic neural systems in PD: non-invasive VNS (nVNS), transcranial direct current stimulation (tDCS), transcranial magnetic stimulation, and caloric vestibular stimulation in PD. Given the increasing recognition that the vagus nerve can also be mediated by ACh-dependent microbiome and SCFA modulations, a brief overview of GBA physiology and its potential for therapeutical modulation will be reviewed. Lastly, the potential of visual rehabilitation approaches to promote neuroplasticity in the hypocholinergic visual thalamus in PD will be discussed.

Non-invasive VNS: The vagus nerve has been recognized as a key and early player in the pathophysiology of PD as an early nidus and propagation vector for spreading of misfolded proteins, such as α-synuclein. The primary neurotransmitter of dorsal motor nucleus (DMN) vagus neurons is ACh. The vagus nerve, the principal component of the parasympathetic nervous system, is a mixed nerve composed of 80–90% afferent and 10–20% efferent fibers. The vagus nerve exits the medulla oblongata through the jugular foramen. The sensory afferent cell bodies are located in nodose ganglia and send information to the nucleus tractus solitarius (NTS). Most organs receive sympathetic efferents through the splanchnic nerves and parasympathetic efferents through the vagus nerve and sacral preganglionic fibers, whether they are in the sympathetic division or in the parasympathetic division, and use ACh as their neurotransmitter. The parasympathetic division (craniosacral outflow) consists of cell bodies from one of two locations: the brainstem (cranial nerves III, VII, IX, and X) or the sacral spinal cord (S2, S3, and S4). The sympathetic division (thoracolumbar outflow) consists of cell bodies in the lateral horn of the spinal cord (intermediolateral cell columns) from T1 to L2. Preganglionic parasympathetic neurons originating from the sacral intermediolateral cell column and the DMN of the vagus, project to myenteric and submucosal neurons.

VNS may affect autonomic functions (either by modulation of the central autonomic network, or peripheral parasympathetic nerves). Potential benefits from VNS may be mediated by activation of connected brain regions, upregulated cholinergic neurotransmission, modulation of non-cholinergic neurotransmitter systems and neuroinflammation, reduced α-synuclein aggregation, and increased neurotrophic factor signaling (Sigurdsson et al., 2021). As an example of connected brain regions, several regions of the forebrain that contain cholinergic neurons or receive dense cholinergic projections from the NBM, including the bilateral dorsolateral prefrontal cortex, caudate nucleus, thalamus, visceral area of the postcentral gyrus, and cerebellum may be activated during VNS as well as in the lower brainstem NTS, parabrachial complex, substantia nigra and ventral tegmental area. VNS may also modulate locus ceruleus-norepinephrine (LC-NE) neurons via afferent projections from the NTS and in turn, may reach the cholinergic NBM. Vagal efferents originate in the DMN of the vagus. Non-invasive VNS is increasingly being used as a research tool to manage DOPA-resistant motor or non-motor symptoms in PD (Morris et al., 2019). Recent studies, even at a single dose level, show promising results to improve DOPA-resistant gait characteristics in PD in early randomized sham-controlled phase 2 clinical trials [for review see Sigurdsson et al. (2021); **Figure 1**]. Basic mechanistic nVNS insights, in particular effects of stimulation frequencies, intensity, pulse width, waveform shape, cycle duration, and how these may affect clinical functions, remain poorly understood (Sigurdsson et al., 2021). Therefore, the evidence for nVNS in PD is promising but limited and further studies are needed.

Indirect neurostimulation approaches target hypocholinergic neural system in PD: tDCS, transcranial magnetic stimulation, and CVS: Indirect neurostimulation/modulation approaches may also be used to target hypocholinergic systems in PD (**Figure 1**). This approach is not based on direct stimulation of cholinergic CNS or PNS structures per se but may involve heteromodal transcranial electrical, magnetic or vestibular stimulation or other neuromodulation approaches that can cause increased activation of or blood flow to hypocholinergic brain circuitry or structures. A recent example is the use of tDCS targeting hypocholinergic brain regions in PD as defined by short-latency afferent inhibition (Oh et al., 2022). Anodal tDCS on the M1 brain area resulted in improved parkinsonian motor ratings, while there was direct evidence of a significant relationship between tDCS-induced changes in motor ratings and the

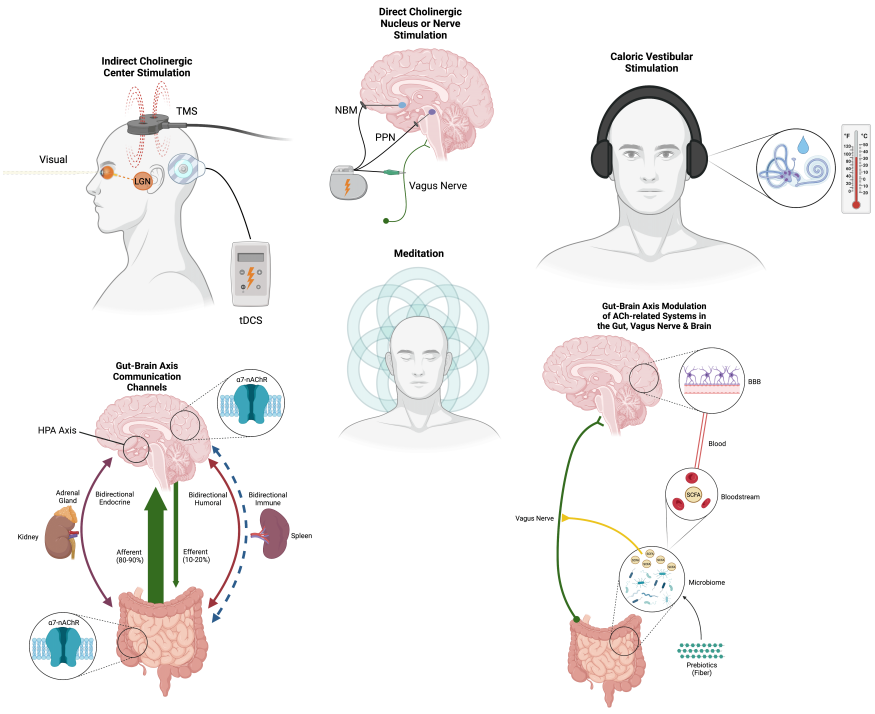

Figure 1 | Indirect and direct neurostimulation/modulation approaches targeting CNS and peripheral cholinergic systems.
TMS, tDCS, CVS, and visual rehabilitation are indirect stimulation approaches that have the potential to target hypocholinergic CNS areas (left upper image). Examples of direct neurostimulation include NBM and PPN deep brain stimulation and VNS (upper mid image). The vagus nerve is a major communication channel of the GBA. There are also bidirectional humoral, immunological and endocrine communications where ACh plays a role (left lower image). The lower images show therapeutic targets for modulation of the microbiome, gut small chain fatty acids, and α7-nicotinic acetylcholine receptors. α7-nAChR: Alpha 7-nicotinic cholinergic receptors; ACh: acetylcholine; CNS: central nervous system; GBA: gut-brain axis; HPA: hypothalamic pituitary adrenal axis; NBM: nucleus basalis of Meynert; PPN: pedunculopontine nucleus; SCFA: short-chain fatty acids; tDCS: transcranial direct current stimulation; TMS: transcranial magnetic stimulation. Created with BioRender.com.

change in short-latency afferent inhibition potentials in PD.

Another indirect cholinergic neuromodulatory approach is a caloric vestibular stimulation (CVS) approach called thermoneuromodulation that may improve motor and non-motor symptoms in PD by boosting PPN connectivity and increasing blood flow to hypocholinergic vestibular neural network hubs, including the brainstem, vestibular cerebellum, and thalamus. Thermoneuromodulation can be self-administered in home situations. A phase 2 double-blinded, placebo-controlled, randomized study demonstrated significant improvements in not only motor symptoms but also non-motor symptoms (Wilkinson et al., 2019).

The vagus nerve as an important mediator of the so-called gut-brain axis in PD – therapeutic implications for modulating ACh-dependent microbiome and metabolic small chain fatty acid, anti-inflammatory, and anti-stress functions: Emerging recognition of communication pathways between gut and brain functions (so-called gut-brain axis (GBA) through vagus and/or spinal cord, metabolic (e.g., SCFA), neuroendocrine (through the hypothalamic-pituitary adrenal, HPA, axis) and neuroimmunological mechanisms (e.g., the release of cytokines via α7-nAChR) may augur novel therapeutic avenues in PD (**Figure 1**). The brain and the gut communicate in a bidirectional way, through the autonomic nervous system and the circumventricular organs. Hypocholinergic or dysfunctional vagus nerve may promote the pathological spreading of a-synucleinopathy and cause neuroinflammation in PD. Therefore, an emerging and promising neuromodulatory therapeutic approach is cholinergic vagus modulation of GBA functions, especially when there is evidence of hypocholinergic gut and other visceral functions in some PD persons, the so-called 'body-first' PD subtype (Horsager et al., 2020). Targeting hypocholinergic gut and other visceral organs by modulating GBA functions mediated by cholinergic enteric nervous system (ENS) and vagus nerve functions may provide a novel but a yet unproven indirect approach to boost hypocholinergic functions both in the gut and the brain. The ENS arises from neural crest cells of primarily vagal origin and consists of two ganglionated plexuses — the submucosal plexus and the myenteric plexus. Interaction of ENS and the vagal nerve as a part of the CNS leads to a bidirectional flow of information and may be exploited for therapeutic opportunities.

Vagus nerve and small chain fatty acids: The vagus nerve can sense microbiota signals through direct mechanisms. For instance, SCFAs produced by the microbiota activate vagal afferent fibers whereas butyrate, a SCFA produced by microbial fermentation of dietary fibers, can enhance the proportion of cholinergic enteric neurons via epigenetic mechanisms and may also have a direct effect on afferent vagus nerve terminals and thus may modulate NTS and its CNS projections, including the parabracheal nucleus, paraventricular nucleus of the hypothalamus, and amygdala that are part of the central autonomic network (Bonaz et al., 2018; Liu et al., 2018). SCFAs that are not metabolized in the colonocytes can reach the portal circulation and may be metabolized by hepatocytes with a minor fraction of SCFAs reaching the systemic circulation and the brain. The abundant expression of monocarboxylate transporters in endothelial cells might not only facilitate the crossing of the blood-brain barrier by SCFAs but also reflect an important role of these postbiotics in maintaining its integrity.

Cholinergic anti-inflammatory effects: Cholinergic DMN neurons together with ACh play a key role in inhibiting neuroinflammation via the cholinergic anti-inflammatory pathway. Immune cells (e.g., macrophages, lymphocytes) produce cytokines, which can lead to afferent signaling through the vagus nerve to the central autonomic network. Subsequent efferent vagal signaling can lead to downregulation of inflammatory cytokines. This occurs in part through the release of ACh in the celiac ganglion, which causes the sympathetic-splenic nerve to release norepinephrine in the spleen. Norepinephrine leads to the production of ACh by T-immune cells, which acts on splenic macrophages that via α7-nAChR dependent processes reduce the production of cytokines. Therefore, VNS may boost the cholinergic system and modulate neuroinflammation in PD. VNS may also modulate heart rate variability, which is inversely related to the levels of inflammatory markers (Sigurdsson et al., 2021).

Vagus nerve and stress responses: The vagal afferent pathways are involved in the activation/regulation of the HPA axis, which coordinates the adaptive responses of the organism to stressors. Stress and cytokines activate the HPA axis through hypothalamic secretion of the corticotropin-releasing factor. Stress inhibits vagus nerve functions and may accelerate vagus-mediated disease processing in PD. Therefore, stress-reducing techniques, such as mindfulness breathing, have the potential to promote vagus nerve function in PD.

Visual rehabilitation approaches to promote neuroplasticity in the hypocholinergic visual thalamus in PD: Vulnerability of the cholinergic lateral and medial geniculate nuclei (metathalamus) is associated not only with the presence of postural imbalance but also with prominent cognitive deficits that are dependent on visual processing. Given increasing recognition of a relationship between poor visual functions and atrophy in the metathalamus there is a need to investigate novel visual rehabilitation strategies to promote neuroplasticity in these key cholinergic hubs. This may be achieved by virtual or augmented reality approaches.

Conclusions and current progress in the field: We conclude that several neuromodulatory approaches with the potential to target hypocholinergic brain or gut regions in PD have gained interest. Apart from current cholinergic pharmacotherapy, there are converging lines of invasive and non-invasive stimulation and modulation approaches that deserve further research as therapeutic options to target the "malignant" hypocholinergic disease phenotype in PD and provide new options to treat DOPA-resistant mobility disturbances and dementia. Direct invasive neurostimulation of cholinergic projections nuclei (NBM, PPN) has shown proof of concept in human studies but requires more refined trials. Phase 2 clinical non-invasive VNS trials show encouraging results for the management of DOPA-refractory gait disturbances. Human VNS trials also have shed light on a multitude of mechanistic effects that by themselves may provide therapeutic opportunities as the vagus is at the interface of the GBA. Of particular interest is vagus nerve modulation of the GBA with postbiotics or VNS that may have profound anti-inflammatory effects not only in the gut but also in the brain through its efferent and afferent fibers and as such may have the potential to modify the disease course in PD. Similarly, the use of α7-nAChR drugs that are currently in clinical trials for inflammatory bowel disease may have potential applications not only to treat visceral but also CNS inflammation in PD or related neurodegenerative disease (e.g., dementia with Lewy bodies). Given the multitude of cholinergic targets and mechanisms at play, multimorbidity, aging changes, and the interplay of additional pathologies, a personalized cholinergic treatment plan should be considered. A more personalized and targeted approach would also avoid a potential 'cholinergic overdose' due to cholinergic compensation, that may occur in specific brain regions before symptomatic deficits (Legault-Denis et al., 2021). Cholinergically mediated clinical measures, such as the presence of falls, dream enactment behaviors, step time variability, or step length variability, may help to implement validated cholinergic augmentation approaches in clinical practice.

Supportive evidence for these non-invasive stimulations and neuromodulatory approaches is most promising for caloric vestibular stimulation (current phase 3 clinical trial), followed by several phases 2 nVNS and microbiome clinical trials, and then followed by DBS NBM and PPN, tDCS and transcranial magnetic stimulation clinical trials (predominant phase 1b and 2a clinical trials). Our center recently launched a phase 2 SCFA clinical trial in PD. There is also a phase 1 clinical trial for visual rehabilitation and neuroplasticity in PD.

Limitations: Limitations of this perspective are that proposed therapeutic approaches in part may be based on hypothesized but not yet fully confirmed assumptions. These assumptions are that cholinergic hypofunction of the gut in PD, including vagal dysfunction, in the presence of α-synuclein aggregation may be partially reversible and that cholinergic hypofunction in the brain is associated with more severe neuroinflammation.

This work was supported by the National Institute of Health, No. P50 NS123067, and The Farmer Family Foundation. Dr. Alison Yarnall is supported by the NIHR Newcastle Biomedical Research Centre (BRC). The NIHR BRC is a partnership between Newcastle Hospitals NHS Foundation Trust and Newcastle University, funded by the National Institute for Health and Care Research (NIHR). The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care. The authors thank Mr. Stiven Roytman for graphical support.

Nicolaas I. Bohnen*, Alison J. Yarnall
Departments of Radiology and Neurology, University of Michigan, Ann Arbor VAMC, Ann Arbor, MI, USA (Bohnen NI)
Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, UK (Yarnall AJ)
***Correspondence to:** Nicolaas I. Bohnen, MD, PhD, nbohnen@umich.edu.
https://orcid.org/0000-0003-0352-6414 (Nicolaas I. Bohnen)
Date of submission: May 9, 2022
Date of decision: June 28, 2022
Date of acceptance: July 20, 2022
Date of web publication: September 16, 2022

https://doi.org/10.4103/1673-5374.353490
How to cite this article: *Bohnen NI, Yarnall AJ (2023) Emerging non-invasive therapeutic approaches targeting hypocholinergic neural systems in Parkinson's disease. Neural Regen Res 18(4):809-810.*
Availability of data and materials: *All data generated or analyzed during this study are included in this published article and its supplementary information files.*

Open peer reviewer: *Jianshe Wei, Institute for Brain Sciences Research, Henan University, China.*
Additional file: *Open peer review report 1.*

References

Bohnen NI, Kanel P, Muller M (2018) Molecular imaging of the cholinergic system in Parkinson's disease. Int Rev Neurobiol 141:211-250.
Bohnen NI, Yarnall AJ, Weil RS, Moro E, Moehle MS, Borghammer P, Bedard MA, Albin RL (2022) Cholinergic system changes in Parkinson's disease: emerging therapeutic approaches. Lancet Neurol 21:381-392.
Bonaz B, Bazin T, Pellissier S (2018) The vagus nerve at the interface of the microbiota-gut-brain axis. Front Neurosci 12:49.
Horsager J, Andersen KB, Knudsen K, Skjærbæk C, Fedorova TD, Okkels N, Schaeffer E, Bonkat SK, Geday J, Otto M, Sommerauer M, Danielsen EH, Bech E, Kraft J, Munk OL, Hansen SD, Pavese N, Göder R, Brooks DJ, Berg D, et al. (2020) Brain-first versus body-first Parkinson's disease: a multimodal imaging case-control study. Brain 143:3077-3088.
Legault-Denis C, Aghourian M, Soucy JP, Rosa-Neto P, Dagher A, Wickens R, Bedard MA (2021) Normal cognition in Parkinson's disease may involve hippocampal cholinergic compensation: An exploratory PET imaging study with [^{18}F]-FEOBV. Parkinsonism Relat Disord 91:162-166.
Liu H, Wang J, He T, Becker S, Zhang G, Li D, Ma X (2018) Butyrate: a double-edged sword for health? Adv Nutr 9:21-29.
Morris R, Yarnall AJ, Hunter H, Taylor JP, Baker MR, Rochester L (2019) Noninvasive vagus nerve stimulation to target gait impairment in Parkinson's disease. Mov Disord 34:918-919.
Oh E, Park J, Youn J, Jang W (2022) Anodal transcranial direct current stimulation could modulate cortical excitability and the central cholinergic system in akinetic rigid-type Parkinson's disease: Pilot study. Front Neurol 13:830976.
Sigurdsson HP, Raw R, Hunter H, Baker MR, Taylor JP, Rochester L, Yarnall AJ (2021) Noninvasive vagus nerve stimulation in Parkinson's disease: current status and future prospects. Expert Rev Med Devices 18:971-984.
Wilkinson D, Podlewska A, Banducci SE, Pellat-Higgins T, Slade M, Bodani M, Sakel M, Smith L, LeWitt P, Ade K (2019) Caloric vestibular stimulation for the management of motor and non-motor symptoms in parkinson's disease: Intention-to-treat data. Data Brief 25:104228.

P-Reviewer: Wei J; C-Editors: Zhao M, Liu WJ, Wang Lu; T-Editor: Jia Y