ORIGINAL ARTICLE

Correlation between Heart Rate Variability and Claustrum Stimulation – Hypothesis, Experimental Studies and Future Perspectives

Bogdan PAVEL[1,2], Mihaela Roxana POPESCU[3], Carmen-Denise-Mihaela ZAHIU[1], Patricia Demetria POPOVICI[1], Diana-Andreea ILIE[1], Madalina GHERGHE[1], Leon ZAGREAN[1], Ana-Maria ZAGREAN[1]

ABSTRACT

Heart rate variability (HRV) has long been associated with cardiovascular risk, especially after a myocardial infarction, but also in general. HRV reflects and is used as a surrogate for the balance between sympathetic and parasympathetic systems in modulating the cardiovascular activity. A low HRV, traditionally associated to sympatho-vagal imbalance, is associated with a worse cardiovascular prognosis. Deep brain stimulation (DBS) is a surgical technique used for severe cases of Parkinson's disease and other neurologic pathologies. DBS is performed in various areas of the brain and through different protocols. The claustrum, an area located between the external capsule and the insular cortex, was recently shown to be connected to Parkinson's motor symptoms. As DBS in other regions of the brain has proven non-motor effects, like influencing the HRV, we sought to document the effect of claustrum stimulation on the sympatho-vagal balance (SVB). Our preliminary data indicates that claustrum stimulation inclines the SVB toward the latter, but more studies are required to observe the long-term effects of this type of stimulation.

Keywords: sympatho-vagal balance, cardiovascular risk, deep brain stimulation, Parkinson's disease, orthostatic hypotension.

REZUMAT

Variabilitatea ritmului cardiac (HRV) este de mult timp asociată cu riscul cardiovascular, mai ales după un infarct miocardic, dar și în general. HRV este utilizată ca surogat pentru obiectivarea echilibrului dintre sistemele simpatic și parasimpatic în modularea activității cardiovasculare. O HRV scăzută este asociată cu un prognostic cardiovascular nefast. Stimularea profundă a creierului (DBS) este o tehnică chirurgicală utilizată pentru cazurile severe de boală Parkinson și alte patologii neurologice. DBS se efectuează în diferite zone ale creierului și prin diverse protocoale. Claustrumul, o zonă situată între capsula externă și cortexul insular, a fost recent asociată cu simptomele motorii ale bolii Parkinson. Deoarece DBS în alte regiuni ale creierului a determinat efecte non-motorii, cum ar fi influențarea HRV, am căutat să documentăm efectul stimulării claustrumului asupra balanței simpato-vagale (SVB). Datele noastre preliminare indică faptul că stimularea claustrumului înclină echilibrul simpato-vagal către acesta din urmă, dar sunt necesare mai multe studii pentru a observa efectele pe termen lung ale acestui tip de stimulare.

Cuvinte cheie: balanța simpato-vagală, risc cardiovascular, stimulare cerebrală profundă, boala Parkinson, hipotensiune ortostatică.

[1] Division of Physiology and Neurosciences, Department of Functional Sciences, „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

[2] Department of Anesthesia and Intensive Care, Clinical Emergency Hospital of Plastic Surgery and Burns, Bucharest, Romania

[3] Department of Cardiology, „Elias" Emergency University Hospital, „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

Contact address:

Mihaela Roxana POPESCU, Department of Cardiology, „Elias" Emergency University Hospital, „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.

E-mail: mihaela-roxana.popescu@umfcd.ro

1. INTRODUCTION

Since Kleiger et al. described the association between heart rate variability (HRV) and increased mortality after myocardial infarction (MI)[1], HRV has frequently been used as a mortality predictor or surrogate index for outcomes in clinical trials regarding secondary prevention in cardiovascular disease (CVD)[2-4]. In patients with CVD, all-cause death and cardiovascular events are linked to lower HRV, characteristic of sympathetic-parasympathetic imbalance[5]. Moreover, even in patients without baseline CVD, lower HRV was associated to a higher lifetime risk of CVD[6], as cardiac autonomic dysfunction in itself is considered a risk factor for CVD[7].

Deep brain stimulation (DBS) is a surgical technique that consists in the electrical stimulation of specific brain structures through a chronically stereotactic implanted electrode. DBS was approved as a therapeutic option in several neurological movement disorders such as Parkinson's disease or parkinsonism[8], essential tremor[9] and dystonia[10]. However, it is reserved for severe cases that are unresponsive to drug-therapy. Also, this technique seems to have promising results in treating epileptic seizures[11], and psychiatric pathologies like obsessive-compulsive disorder[12], severe treatment-resistant depression[13], Tourette's syndrome, polydipsia and eating disorders[14].

During DBS, the sympatho-vagal balance (SVB) can be impacted, with an effect on the cardiovascular regulation, making DBS a topic of interest for physiologists, neurologists, neurosurgeons and cardiologists alike.

In this article, we explore the existing data in the literature connecting the stimulation of deep brain areas, such as the claustrum, in neurological diseases and the effect on the SVB and further, on cardiovascular risk and disease.

2. THE EFFECT OF DEEP BRAIN STIMULATION ON THE SYMPATHO-VAGAL BALANCE, ASSESSED BY HEART RATE VARIABILITY

Heart rate varies in response to different physiologic factors as dictated by the autonomic nervous system through a complex balance between the parasympathetic and sympathetic systems[15]. This variation of consecutive RR intervals on the electrocardiogram (ECG) is known as heart rate variability (HRV). Thus, HRV is a convenient tool for assessment of the SVB.

Heart rate normally varies cyclically, even at rest, with the most significant variation happening between day and night[16]. Other fluctuations of the HRV happen due to baroreflexes, thermoregulation or are modulated by ventilation, exercise or work-related stress[7,15]. By means of the Fourier transformation, these fluctuations are depicted by high frequency (HF) fluctuations, ranging between 0.15 Hz - 0.40 Hz, and low frequency (LF) fluctuations, ranging between 0.04 Hz - 0.15[15]. Commonly, HF is attributed to vagal predominance and LF to both vagal and sympathetic influences. Also, a LF/HF ratio can be calculated in order to evaluate the state of the SVB.

A reduced HRV is linked to cardiovascular risk factors like sedentary lifestyle, diabetes, hypertension, and CVD[6], and reveals a disruption of the SVB, either through reduced vagal activity or increased sympathetic activation.

The SVB can be also disrupted in neurological diseases associated with autonomic dysfunction, like Parkinson's disease (PD), traumatic brain injury, dementia, etc.[17-19]. Moreover, the SVB is impacted in DBS-type interventions used in neurological diseases, like PD.

Data related to subthalamic stimulation is controversial and its effect on SVB is still unclear. Subthalamic stimulation for PD was not associated with an improvement in the cardiovagal control of patients with advanced disease in some studies[20,21], while in others it was shown to improve orthostatic hypotension without influencing the heart rate[22]. However, in other reports, this type of stimulation influences both heart rate and blood pressure through sympathetic stimulation. Recent studies confirmed that DBS of the subthalamic nuclei is able to improve cardiovascular health rather through increased mobility than a direct effect on the autonomic nervous system[23]. Pallidal and insular stimulation were also linked to a cardiovascular modulation effect[21].

3. DEEP BRAIN STIMULATION AND CLAUSTRUM STIMULATION

The claustrum is a small cerebral structure located between the external capsula and the insular cortex, containing mostly excitatory neurons. The claustrum is connected with almost all cerebral cortical and subcortical structures, mainly by efferent projections. It is supposed to be involved in conscious states, as awareness, anesthesia and possibly epilepsy[24,25]. This assumption is sustained by the in vivo stimulation of the claustrum that associates deepening of different

conscious states, as it happens in awake or anesthetized subjects[24,25].

DBS is a useful tool in management of severe cases of different neurological diseases, as PD, etc. Recently, the claustrum was connected to Parkinsonism's tremors, rigidity and slowed movement[26]. This new perspective on Parkinson's location of focal brain lesions causing the disease's specific symptomatology, indicate the claustrum as a novel treatment target for intervention. Thus, claustrum stimulation in PD should also explore the effects on the autonomic nervous system, as it is known that PD is associated with signs of autonomic dysfunction (neurogenic orthostatic hypotension, supine hypertension, postprandial hypotension)[17].

DBS uses either bipolar or monopolar implanted electrodes to deliver electrical pulses characterised by frequency, voltage, current intensity and pulse width. There are high variation between studies in DBS parameters, mainly in the current intensity and the pulse frequency values, ranging between 0.86 μA -1100 μA, and 1 Hz-1000 Hz, respectively[27].

DBS using frequencies above 100 Hz is called high frequency stimulation (HFS). This type of overstimulation is considered to produce inhibition of neuronal activity in the target brain region[27]. In PD patients, HFS of subthalamic nucleus (STN) alievates the motor symptoms of rigidity, tremor, and bradykinesia, similar to surgical destruction of this nucleus. *In vitro* electrophysiological studies and *in vivo* electrophysiological recordings in PD rat-models showed that HFS of STN blocked the spontaneous discharges of STN neurons in a frequency-dependent maner. Thus, the inhibition increases gradually with the stimulation frequency, and the local neuronal activity is completley blocked at frequencies of 166–250 Hz[27-29].

Choosing the place of DBS depends on the neural circuits involved in the pathophysiology of the neurological disease, and multiple stimulation sites are proposed for each pathology, as shown in Table 1.

The current algorithms for programming DBS start with conventional settings that are further adjusted based on the amelioration of symptoms in the absence of side effects[33]. Current research focuses on the devellopment of adaptative DBS techniques that can modulate DBS parameters based on electrophysiological biomarkers, local field potentials (LFP), LFP – electromiography coherence or neural oscilatory activity[34-36].

4. HYPOTHESIS: CLAUSTRUM STIMULATION IS ASSOCIATED WITH A PARASYMPATHETIC ACTIVATION

The claustrum has an excitatory role in the human brain (80% of neurotransmitters are excitatory)[37] and inhibition of the claustrum during anesthesia[38] or possible through electrical stimulation[24], leads to decreased cortical activity. Thus, we considered clinically important to explore the effect of claustrum inhibition on SVB. Furthermore, we wanted to check if this inhibition is accompanied by decreased sympathetic activity/increased vagal dominance.

The changes in SVB induced by claustrum inhibition (via electrical stimulation) might be clinically relevant, as patients who have brain stimulation procedures for neurological disorders occasionally associate ischemic heart disease. More to this point, it is already known that secondary to brain trauma or stroke, cardiac ischemia by sympathetic overstimulation can ensue[39]. Further activation of the sympathetic nervous system could lead to an unwanted coronary ischemic event.

Exploring the claustrum activity, through its connections with most of the brain structures, could be a good experimental model for a global assessment of the effect of brain stimulation on the SVB. In our preliminary study, which we will detail further on, we investigated the effect of stimulating the anterior portion of the claustrum on the SVB.

Table 1. The brain regions targeted by deep bran stimulation (DBS) in neurological disorders (in bold, the prefered brain regions)

Disease	The neural structures targeted by DBS
Parkinson's disease	**STN, GPi,** PPN, VIN[30]
Dystonia (generalised and focal)	**GPi,** VIN, STN[30,31]
Essential tremor	**VIM,** STN[30,32]
Epilepsy	**ATN,** CMTN, hippocampus, BG (CN, STN), posterior hypothalamus and cerebellum[11,30]

ATN = anterior thalamic nucleus, CMTN = centromedian thalamic nucleus, BG = basal ganglia, STN = subthalamic nucleus, CN = caudate nucleus, Gpi = globus pallidus internus, PPN = pedunculo pontinue nucleus, VIM = ventral intermediate nucleus of the thalamus

5. PROOF OF CONCEPT, EXPERIMENTAL SETTING AND RESULTS

Material and methods

This study was carried out with local ethical committee approval, in accordance with the recommendations of the European Communities Council Directive 86/609/EEC on the protection of animals used for scientific purposes.

In this study, we used adult male Wistar rats (n = 4) with body weights (BW) of 250-300 gr. The animals were housed under standard conditions with water and food ad libitum at a 12 h light-dark cycle.

Anesthetic Protocol for Surgery

Anesthesia was induced in the animal induction chamber (Classical T3 Vaporizer, SurgiVet, USA) using 2.5% isoflurane (Abbott Laboratory, USA), with a delivery rate of 0.8 l/min for 5 min. Chloralhydrate 400 mg/kg or a ketamine-xylazine cocktail (90 mg/kg BW ketamine and 10 mg/kg BW xylazine) was administered by intraperitoneal route, for pain control.

Surgical Technique and Electrode Placement for Claustrum Stimulation

A median line incision starting from the half of the interocular line, leading to the half of the interauricular line was performed. The scalp and the periosteum were dissected. Four small craniotomies were generated with a precision drill (FBS 240/E, Proxxon Micromot), corresponding to the coordinates of left claustrum, the frontal cortex, the ipsilateral parietal cortex and the reference electrode, as previously described[24]. Other two recording electrodes were placed on the anterior paws, and one ground electrode on the left posterior paw, in order to record the ECG tracing with BIOPAC MP150 Systems (BIOPAC Systems Inc., USA). All the electrodes were handmade in our laboratory. For EcoG recording electrodes we used Ni-Cr wires and for stimulation electrodes Wolfram wires were used. At the end of experiment, the stimulation area was checked by gentian violet injection through a glass cannula placed using the stimulating electrode's stereotactic coordinates (see Figure 1). The rats were then sacrificed and perfused with formaldehyde. Brains were removed and preserved for another 24 h in formaldehyde at 4°C, then included in agarose (2%) and sliced into 100 µm coronal sections using a vibratome (Leica VT 1000S).

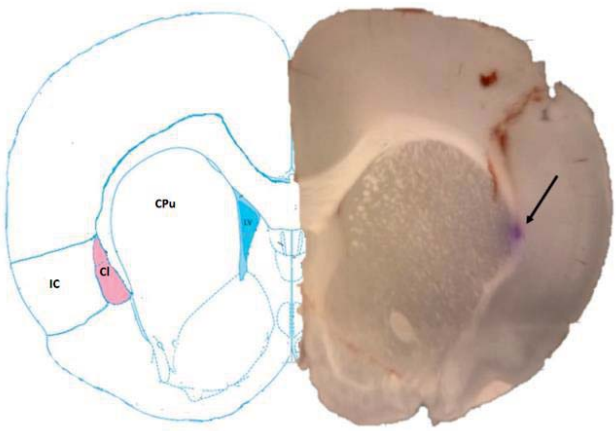

Figure 1. The right part of the figure represents an example of gentian-violet staining indicating the stimulation site (coronal section at 1.8 mm anterior to bregma; optic microscope photo, 4× magnification). The black arrow indicates the claustrum. In the left part of the figure, a simplified diagram of the corresponding rat brain structures, modified after Paxinos atlas[40]. Cl = claustrum; CPu = caudate putamen; IC = insular cortex, LV = lateral ventricle.

Electrocorticogram Recording and Claustrum Stimulation Protocol

Acquisition of the electrocorticogram (ECoG) signal (high pass 30 Hz) was performed using a BIOPAC MP150 Systems. ECoG was also recorded in order to observe the cortical activity changes secondary to stimulation (data not shown here).

Through a bipolar electrode stereotactically introduced in the left claustrum, at a depth of 5.5 mm from the cortical surface, ten trains of rectangular stimuli (0.2 ms pulse width at 50 Hz and 800 µA intensity) of 5 s duration each were applied at an interstimulus interval of 5 s in one single recording session, using a stimulation device (A395 Linear Stimulus Isolator, WPI). In order to evaluate the electrical response of claustrum's surrounding regions to the same stimulation protocol, the electrode was carefully repositioned from -5.5 mm, to 3 mm upward (at 5.2 mm from the cortical surface) and downward (at 5.8 mm from the cortical surface), and finally, at 1.2 mm in the cerebral cortex. A stabilization time period of 2 min between these stimulation sessions was used. At -5.5, two stimulation sessions were performed, to be certain about the claustrum response. The placement of the stimulation electrode was checked post factum under the microscope (in gentian violet staining) in the correspondent brain coronal sections, obtained as described above.

ECG Data Analysis

In order to analyze ECG, we used the recordings saved as *.acq files. The AcqKnowledge 4 software has been used for computing the heart rate variability. (https://www.biopac.com/wp-content/uploads/acqknowledge-

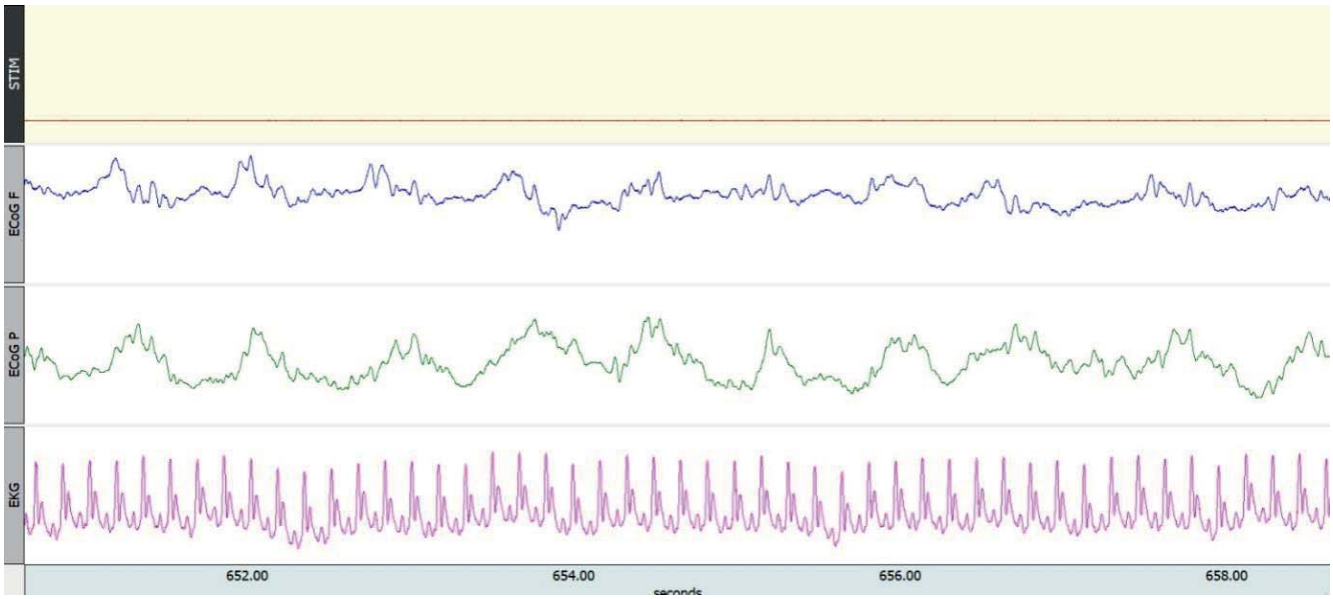

Figure 2. The electrocortical (ECoG) and electrocardiogram (ECG) activity during basal condition (no stimulation). The first channel (red) is represented by the raw stimulation, the second channel (blue) recorded the electrocortical activity on the frontal lobe, the third channel (green) recorded the electrocortical activity on the parietal lobe, the fourth channel (magenta) recorded the electrocardiogram (ECG).

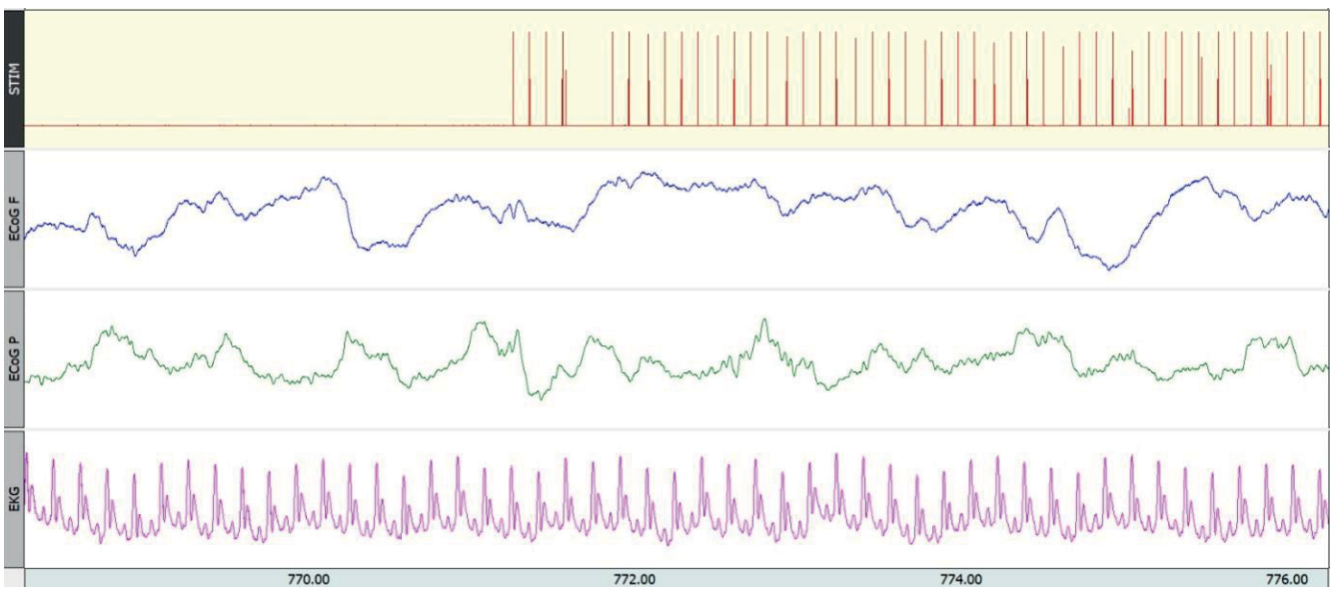

Figure 3. The electrocorticogram (ECoG) and electrocardiogram (ECG) activity during stimulation. The first channel (red) is represented by the raw stimulation, the second channel (blue) recorded the electrocortical activity on the frontal lobe, the third channel (green) recorded the electrocortical activity on the parietal lobe, the fourth channel (magenta) recorded the electrocardiogram (ECG).

4-software-guide.pdf). This software is based on the algorithm presented in: Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology[41]. For the HRV analysis, the Frequency Bands were set as follows: very low frequency band (0 Hz to 0.04 Hz), Low frequency band (0.04 Hz to 0.6 Hz), High frequency band (0.6 Hz to 1.4 Hz) and Very high frequency band (1.4 Hz to 3 Hz). The analysis was performed by dividing the entire recording into 6 segments of 120 sec each, corresponding to: pre-stimulation, the first stimulation at -5.5 mm, the second stimulation at -5.5, stimulation at -5.8, -5.2 and respectively, at -1.2. We reported the sympathetic activity, parasympathetic (vagal) activity

Table 2. The effects of claustrum electrical stimulation on the sympatho-vagal balance

Anesthetic	Basal S/V	S1/V 1 - 5.5	S2/V 2 - 5.5	S/V -5.8	S/V -5.2	S/V -1.2
Xyl-Ket	2,35	0,69	0,66	0,67	0,76	0,85
Xyl-Ket	6,56	1,87	0,64	0,46	2,07	2,61
Xyl-Ket	0,94	0,34	0,41	0,23	0,65	0,33
CHL	2,78	0,46	0,52	0,91	0,68	0,63

Basal S/V – sympathetic-vagal balance under basal condition. S1/V1 -5.5 - sympathetic-vagal balance during the first stimuli at -5.5 mm. S2/V2 -5.5 - sympathetic-vagal balance during the second stimuli at -5.5 mm. S/V -5.8 - sympathetic-vagal balance during the stimulation at -5.8 mm. S/V -5.2 - sympathetic-vagal balance during the stimulation at -5.2 mm. S/V -1.2 - sympathetic-vagal balance during the stimulation at – 1.2 mm. Xyl-Ket – xylazine-ketamine, CHL – chloral hydrate.

and SVB before stimulation and at each level of stimulation. Also, there is great interindividual variability under anesthesia in rats, so we considered each rat as its own control.

RESULTS

Our data have shown a great variability of the SVB between rats in basal conditions (from 6.6 to 0.94). There is an increase in vagal dominance during stimulation regardless of the stimulated area in all the subjects. The highest values of vagal activity were obtained during stimulation in the claustrum area at -5.5 mm, -5.8 mm and -5.2 mm, respectively. The recorded values were between 0.34-1.87 during the first stimulation and between 0.41-0.66 during the second stimulation at - 5.5 mm. Stimulation at -5.8 mm yielded values between 0.23-0.91, while for the following depths of -5.2 mm and -1.2 mm, we logged values of 0.65-2.07 and 0.33-2.61 respectively (see Table 2 and Annex 1).

6. DISCUSSION AND PERSPECTIVES

Our pilot study focuses on claustrum, a brain structure less investigated in DBS protocols, but that seems to be a promissing target for drug-resistant epilepsy and PD[25,26], diseases frequently associated with cardiac autonomic dysfunction[42]. These preliminary data revealed an increased vagal activity during claustrum stimulation and in some circumstances, in the surounding areas, as suggested by the decrease in the SVB balance compard to basal activity. These data do not allow the claim that specific stimulation of the claustrum or global cerebral and cortical stimulation are responsible for increased vagal activity. However, even non-specific stimulation in certain areas causes increased vagal activity associated with better outcomes in neurological disease requiring DBS.

Although claustrum is not a component of the central autonomic network, our data indicate that its stimulation can modulate HRV. This could be partially explaind by the location of the claustrum adjacent to the insular cortex[43], and the connections between the claustrum and amygdala. The insular cortex is a structure conected with the amygdala, hypothalamus, periaqueductal gray matter, parabrachial complex, ventral medulla and the nucleus tractus solitarus, forming the central autonomic network[44]. The insula is involved in cardiovascular activity regulation, as sustained by lesion and stimulation studies in animal models and humans. These studies revealed increased sympathetic responses during stimulation of the right rostral insular cortex. Additionally, stimulation of the left insula and caudal right insula caused depressed cardiomotor function with decreased blood pressure and bradycardia[45,46]. Further studies are needed to assess the excitatory or inhibitory neural effects of claustrum stimulation.

To the best of our knowledge, there are missing clinical randomised control trials to compare different stimulation protocols and to find the optimal parameters of DBS for a specific target. We chose to use a high-frequency stimulation for the claustrum based on previous stimulation protocols[25,27-29]. Also, our study focused on a single acute stimulation session-induced HRV changes. However, the DBS in Parkison's disease is a chronic procedure, with long-term effects as shown by the 2, 5 or 7 years follow-up studies[47,48]. Further study on the long-term effect of claustrum stimulation is the next logical step in this scientific endeavour.

While reduced sympathetic activity is connected to less cardiovascular risk, PD is associated with neurogenic orthostatic hypotension caused by both baroreflex failure and sympathetic denervation[17]. Thus, it remains to be clarified by further studies if DBS is a friend or foe in patients with PD and which region should be stimulated for best results. Also, due to their motor symptoms, post-MI PD patients cannot undergo exercise training, shown to be beneficial in improving the

post MI disrupted SVB[49]. Thus, if these patients were DBS candidates, they might benefit more from DBS than just for their motor symptoms, through potential improvement of the SVB and consequent cardiovascular risk reduction.

Compliance with ethics requirements:

The authors declare no conflict of interest regarding this article. The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

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APPENDICES

Annex 1. The effects of claustrum electrical stimulation on sympathetic, vagal activity and sympatho-vagal balance

Anesthetic	Basal S	Basal V	Basal S/V	S 1 -5.5	V 1 -5.5	S1/V 1 - 5.5	S 2 -5.5	V 2 -5.5	S2/V 2 - 5.5	S -5.8	V -5.8	S/V -5.8	S -5.2	V -5.2	S/V -5.2	S -1.2	V -1.2	S/V -1.2
Xyl-Ket	0,7	0,29	2,35	0,4	0,59	0,69	0,39	0,6	0,66	0,4	0,59	0,67	0,43	0,56	0,76	0,45	0,54	0,85
Xyl-Ket	0,9	0,13	6,56	0,65	0,34	1,87	0,39	0,6	0,64	0,31	0,68	0,46	0,67	0,32	2,07	0,72	0,27	2,61
Xyl-Ket	0,5	0,51	0,94	0,25	0,74	0,34	0,29	0,7	0,41	0,18	0,81	0,23	0,39	0,6	0,65	0,25	0,74	0,33
CHL	0,7	0,26	2,78	0,31	0,68	0,46	0,34	0,65	0,52	0,47	0,52	0,91	0,4	0,59	0,68	0,38	0,61	0,63

Basal S – sympathetic activity during basal condition, Basal V- vagal activity during basal condition, Basal S/V – sympathetic-vagal balance under basal condition. S1 -5.5 - sympathetic activity during the first pairs of stimuli at -5.5 mm, V1 -5.5 - vagal activity during the first pairs of stimuli at -5.5 mm, S1/V1 -5.5 - sympathetic-vagal balance during the first pairs of stimuli at -5.5 mm. S2 -5.5 - sympathetic activity during the second pairs of stimuli at -5.5 mm, V2 -5.5 - vagal activity during the second pairs of stimuli at -5.5 mm, S2/V2 -5.5 - sympathetic-vagal balance during the second pairs of stimuli at -5.5 mm. S - 5.8 - sympathetic activity during the stimulation at -5.8 mm, V -5.8 - vagal activity during the first pairs of stimuli at -5.8 mm, S/V -5.8 - sympathetic-vagal balance during the stimulation at -5.8 mm. S - 5.2 - sympathetic activity during the stimulation at -5.2 mm, V -5.2 - vagal activity during the first pairs of stimuli at -5.2 mm, S/V -5.2 - sympathetic-vagal balance during the stimulation at -5.2 mm. S – 1.2 - sympathetic activity during the stimulation at – 1.2 mm, V – 1.2 - vagal activity during the first pairs of stimuli at – 1.2 mm, S/V -1.2 - sympathetic-vagal balance during the stimulation at – 1.2 mm. Xyl-Ket – xylazine-ketamine, CHL – chloral hydrate.