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Assessing blink reflex circuits by three different afferent routes in Parkinson's disease

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HIGHLIGHTS

- Establishment of new blink reflex variant by stimulation of the auricular branch of the vagus nerve.
- Testing of blink reflex variants elicited by three different afferents in PD.
- No evidence for malfunctioning of differential neural brainstem circuits in PD.

ABSTRACT

Objective: Degeneration of nuclei of the brainstem, especially parts of the vagal nuclei complex and the reticular formation, in Parkinson's disease (PD) may in part be responsible for nonmotor signs like obstipation, cardiac dysfunction and rapid eye movement sleep behavior disorder (RBD). The aim of the study was to establish a new blink reflex (BR) variant involving the vagal nuclei complex and the reticular formation and to investigate BR comprehensively using 3 different afferent routes in PD.
Methods: In this cross-sectional observational study in 30 PD patients and 30 age and sex matched healthy controls, BR was elicited by stimulation of the auricular branch of the vagus nerve (ABVN) and compared to conventional BR variants evoked by the trigeminal and median nerve.
Results: BRs could be elicited reliably by stimulation of ABVN in both groups. In none of the three BR variants, latencies or amplitudes differed between PD patients and controls. In PD, BR parameters were not related to cognition or presence of RBD.
Conclusion: The present study did not provide evidence for malfunctioning of neural circuits subserving BRs elicited by three different afferents in PD.
Significance: Brainstem circuits mediating these BR variants may be spared from neurodegeneration in PD.

1. Introduction

Degeneration of dopaminergic neurons in the substantia nigra pars compacta remains a pathological hallmark of Parkinson's disease (PD). According to Braak and colleagues, the typical Lewy pathology and neurodegeneration starts in more caudal brainstem structures, namely, the reticular formation (RF) and parts of the vagal nuclei complex such as the dorsal motor nucleus and the visceroafferent solitary nucleus of the vagus nerve (Sulzer and Surmeier, 2013; Braak et al., 2003). From a clinical point of view, degeneration of the RF may be responsible for rapid eye movement sleep behavior disorder (RBD; Boucetta et al., 2016; Iranzo et al., 2013; Scherfler et al., 2011). Affection of the vagal nuclei complex may account for gastrointestinal and cardiac dysfunction, symptoms that often already occur in a premotor period of PD (Klingelhoefer and Reichmann, 2015). The RF is also part of the blink reflex circuit where signals are transmitted through a polysynaptic chain of interneurons (Nieuwenhuijzen et al., 2006; Smit et al., 2006). Spontaneous and reflex blinking is known to be affected in PD and other neurodegenerative parkinsonian syndromes and may show as reduced spontaneous blink frequency (Deuschl and Goddemeier, 1998; Korosec et al., 2006), decreased amplitude and peak velocity of spontaneous blinking (Agostino et al., 2008) and disinhibited glabella reflex (Brodsky et al., 2004). However, previous studies revealed heterogeneous results

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regarding the median or trigeminal nerve evoked blink reflex (BR) in PD and other neurodegenerative diseases (Bonanni et al., 2007; Valls-Solé et al., 1997; Peshori et al., 2001; Cruccu and Deuschl, 2000).

We hypothesized first that a blink reflex can be evoked by stimulation of the auricular branch of the vagus nerve (ABVN). Secondly, as the RF and the vagal nuclei complex are parts of the BR brainstem pathway, we hypothesized that this new ABVN-BR may be more sensitive to brainstem pathology in PD than established BR variants.

2. Subjects and methods

2.1. Standard protocol approvals, registrations, and patient consents

The study was approved by the ethics committee of the Medical Faculty of the University of Leipzig (reference no.: 130-11-18042011) and all participants gave their written informed consent.

2.2. Demographic and clinical data

Thirty patients with a clinical diagnosis of PD according to the British brain bank criteria (Hughes et al., 1992) were recruited from the outpatient clinic at the Department of Neurology of the University of Leipzig. Exclusion criteria were deep brain stimulation, a clinical history of stroke or traumatic brain injury. All patients were on antiparkinsonian medication. As a control group thirty healthy, age and sex matched subjects with normal results on neurological examination were recruited. Exclusion criteria for controls were history of stroke, traumatic brain injury or neurodegenerative disease, and clinical signs of PD. The Montreal Cognitive Assessment was used for the assessment of cognition (Dalrymple-Alford et al., 2010) and the RBD screening questionnaire was applied (Stiasny-Kolster et al., 2007).

2.3. Stimulation and recording

Subjects were seated comfortably in an armchair in a quiet, temperature controlled room (24 °C). They were instructed to gently close their eyes.

For transcutaneous electric stimulation custom-made fine silver wires (ABVN-BR) and a standard stimulation block (bar electrode) connected to a Digitimer DS7A stimulator (Digitimer Ltd., Welwyn Garden City, United Kingdom) were used. For stimulation of the left and right ABVN the stimulation electrodes were attached to the skin of the inner side of the tragus at the outer ventral edge of the external auditory meatus (using electroencephalogram conductive paste). The supraorbital branch of the trigeminal nerve blink reflex (SOTN-BR) and median nerve blink reflex (MN-BR) were stimulated at the supraorbital foramen (cathode caudally) and wrist (cathode proximal), respectively.

The stimulation intensity for ABVN-BR assessment was set at 8-times perceptual threshold (20.0 ± 7.5 mA), 5-times for the SOTN-BR (17.2 ± 4.0 mA) and at 10-times for the MN-BR (50.6 ± 13.6 mA). In preliminary studies on healthy volunteers these intensities were found to lead to reliable results and minimal discomfort.

All BR variants were elicited five times on each side using a stimulus duration of 100 μs and an interstimulus interval of 20 ± 10 s to avoid habituation.

Surface electromyographic activity was recorded from both orbicularis oculi muscles using disposable surface electrodes (Ambu Neuroline 710, Ambu GmbH, Bad Nauheim, Germany) in all BR variants (Fig. 1). Electrodes were placed on the inferior part of the orbicularis oculi muscle on each side and the reference electrode was placed on the forehead.

Raw signals were amplified using a CED 1902 signal conditioner (Cambridge Electronics Design, Cambridge, United Kingdom) and bandpass filtered between 1 Hz and 2 kHz. Electromyographical signals were digitized at 5 kHz by an A/D converter (model 1401 plus, Cambridge Electronics Design, Cambridge, United Kingdom) and stored on a laboratory computer for display and later off-line analysis.

The latencies of the ipsilateral R1 (SOTN-BR) and the ipsilateral R2 and contralateral R2 as well as the R2i and R2c area under the curve (AUC) were determined offline, with the experimenter blinded to the identity and clinical status of the participant, and used for further statistical analyses.

2.4. Statistical analysis

Since data showed a non-normal distribution in the Kolmogorov-Smirnov test and the Shapiro-Wilk test, non-parametric Mann-Whitney-U test was used to test for differences in latencies and AUC between groups. For the comparison of the presence or absence of responses of the ABVN-, the SOTN- and the MN-BR between groups Chi square test was used. In addition, a multiple regression analysis was performed with latencies and AUC of the ABVN-, SOTN- and MN-BR as dependent variable and adjustment for RBD screening questionnaire and the Montreal Cognitive Assessment. A further multiple linear regression analysis was added to examine the selective impact of age, disease duration and the score on the Unified Parkinson's Disease Rating Scale Part 3 on latencies and AUC. All values are given as means ± standard deviation. Effects were considered significant if $p < 0.05$. Statistical analyses were performed with SPSS version 24.0 (IBM Corporation; New York, NY, USA).

3. Results

3.1. Demographic and clinical parameters

Patient and control group were matched in terms of age (63.7 ± 9.2 vs. 62.0 ± 12.1 years) and sex distribution (13 females and 17 males in each group, Supplementary Table S1). Patients with PD had a mean disease duration of 7.5 ± 3.9 years, a mean Unified Parkinson's Disease Rating Scale Part 3 score of 19.9 ± 7.4 and mean levodopa equivalent dose of 744 ± 340 mg. There was no difference in the Montreal Cognitive Assessment (26.9 ± 2.8 vs. 27.7 ± 1.6; Mann-Whitney-U test, $p = 0.482$) and the RBD screening questionnaire (4.9 ± 2.8 vs. 3.3 ± 1.8; Mann-Whitney-U test, $p = 0.062$).

3.2. Presence of response of blink reflexes

The ABVN-BR (R2i and R2c) could be elicited in 26 out of 30 controls (in 22 out of the 26 following stimulation on the right *and* left side, in 4 out of the 26 only following either right *or* left stimulation) and in 26/30 PD patients (in 16/26 following stimulation on the right *and* left side, in 10/26 only following either right *or* left stimulation), the MN-BR in 22/30 controls (in 19/22 following stimulation of the right *and* left side, in 3/22 only following either right *or* left stimulations) and in 24/30 patients (in 15/24 following stimulation on the right *and* left side, in 9/24 only following right *or* left stimulation) and the SOTN-BR in all subjects (in one PD patient only following left stimulation) (Fig. 1). The presence of response of any blink reflex variant did not differ between both groups (each $p > 0.05$; Chi square).

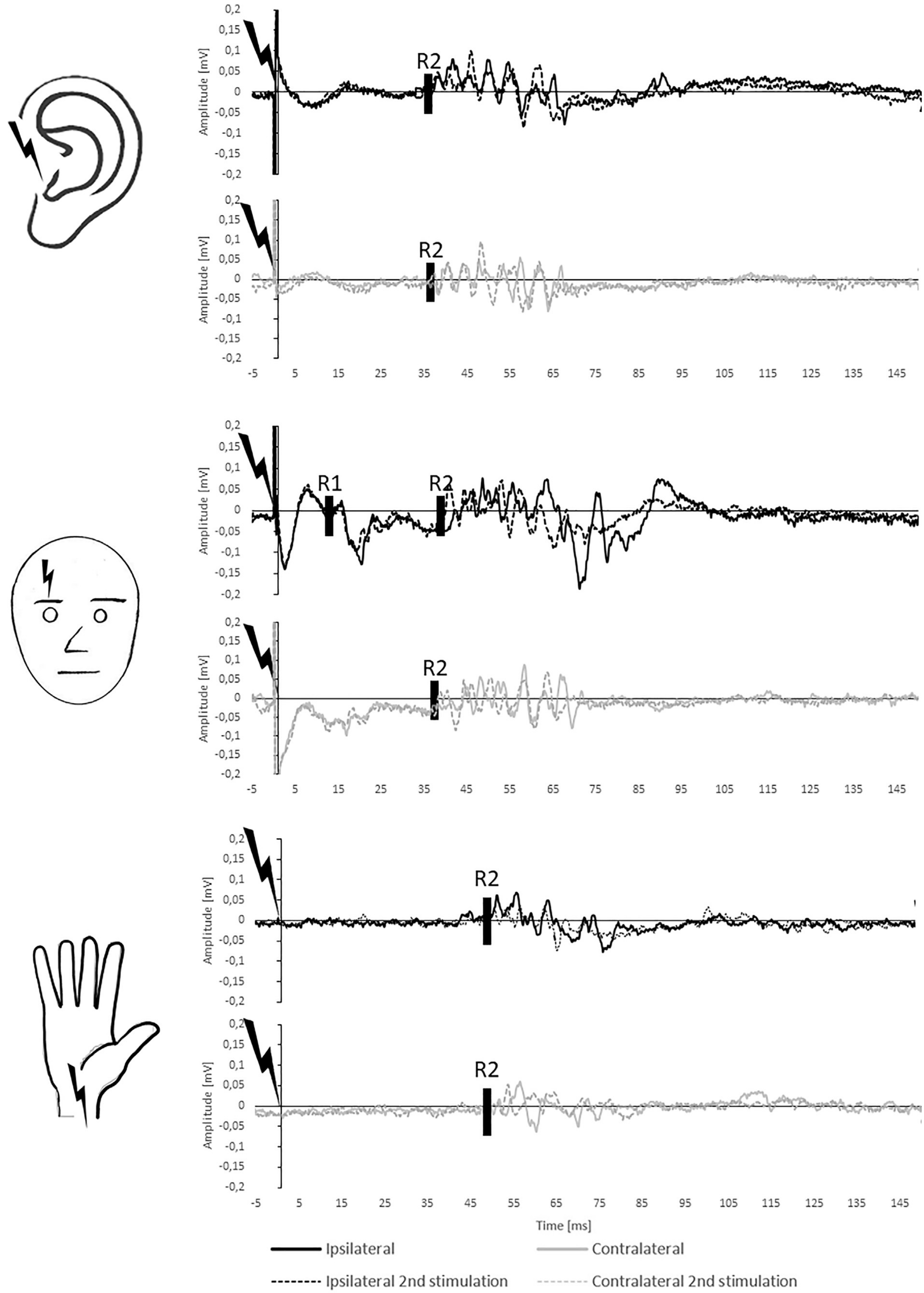

Fig. 1. Examples of the blink reflex after stimulation of the auricular branch of the vagus nerve, supraorbital branch of the trigeminal nerve and median nerve of a representative PD patient.

Table 1
Comparison of R2 latencies ipsi- (R2i) and contralateral (R2c, A) and area under the curve (B) of the blink reflex (BR) after stimulation of the auricular branch of the vagus nerve (ABVN), supraorbital branch of the trigeminal nerve (SOTN) and median nerve (MN) between patients with Parkinson's disease (PD) and control subjects. All values are given as means and standard deviation. All statistical analyses were calculated using the Mann-Whitney-U test.

Blink reflex	Side	R2i [ms]			R2c [ms]		
		PD	Controls	P-value	PD	Controls	P-value
A Latencies							
ABVN	right	45.4 ± 6.8	45.4 ± 8.8	0.83	47.2 ± 7.3	46.0 ± 8.0	0.56
ABVN	left	44.7 ± 6.2	47.2 ± 7.3	0.72	47.3 ± 7.7	45.4 ± 6.8	0.13
SOTN	right	37.8 ± 4.3	39.0 ± 5.0	0.59	37.6 ± 4.0	38.3 ± 5.6	0.43
SOTN	left	37.3 ± 3.1	38.3 ± 5.6	0.60	38.6 ± 4.8	39.0 ± 5.0	0.81
MN	right	60.1 ± 13.8	56.4 ± 7.0	0.51	61.9 ± 13.0	58.2 ± 7.7	0.40
MN	left	58.6 ± 7.4	54.6 ± 4.3	0.08	60.5 ± 8.7	56.9 ± 5.2	0.10
B Area under the curve							
ABVN	right	1141 ± 993	1229 ± 1044	0.991	703 ± 451	1051 ± 1025	0.317
ABVN	left	1080 ± 716	1654 ± 2307	0.804	979 ± 1364	1328 ± 1577	0.349
SOTN	right	2529 ± 2485	2267 ± 1516	0.994	2062 ± 2789	1644 ± 816	0.810
SOTN	left	2049 ± 1214	2128 ± 1275	0.813	1800 ± 1455	1930 ± 1272	0.585
MN	right	1054 ± 763	918 ± 485	0.818	848 ± 719	845 ± 605	0.778
MN	left	1111 ± 1052	952 ± 607	0.866	907 ± 640	864 ± 483	0.918

3.3. Comparison of electrophysiological parameters between patients with Parkinson's disease and healthy controls

Both, the ipsi- and contralateral latencies (R2i and R2c) and the ipsi- and contralateral AUC (R2i and R2c) of the ABVN-, SOTN- and MN-BR were similar between the PD and the control group (Table 1; Mann-Whitney-U, each $p \geq 0.05$). This was confirmed by a multiple regression analysis with adjustment for the RBD screening questionnaire and the Montreal Cognitive Assessment (data not shown). We also found no plausible correlation between latencies and AUC and age, disease duration, Unified Parkinson's Disease Rating Scale Part 3 or RBD screening questionnaire in PD patients (Supplementary Table S2).

4. Discussion

In the present study we introduce a method of eliciting a BR with stimulation of the ABVN as the afferent component. Using different variants of BR testing that included the novel ABVN-BR we did not find any abnormalities regarding latencies or AUCs in a population of moderately affected PD patients. Furthermore, PD patients and healthy controls each with and without RBD did not differ in any BR parameter.

This appears to be the first report describing that a BR may be reliably evoked by electrical stimulation of cutaneous fibres of the ABVN. The classic BR is elicited by supraorbital nerve stimulation and its afferent and efferent pathways are well known (for review, see (Cruccu and Deuschl, 2000)). The reflex circle of the R1 component of the BR consists of the afferent medium-myelinated (A-β) fibers of the supraorbital nerve and is relayed through an oligosynaptic circuit to the facial motoneurons in the pons (Cruccu et al., 2005; Kimura et al., 1994). The R2 BR component is relayed through a more complex route. The afferents of the R2 component are conducted via the spinal tract of the dorsolateral region of the pons and medulla oblongata to the caudal part of the spinal trigeminal nucleus (Ongerboer de Visser and Kuypers, 1978; Kimura and Lyon, 1972; Cruccu et al., 2005). Signals are then transmitted ipsi- and contralaterally through a polysynaptic chain of interneurons belonging to the bulbopontine and pontomedullary RF before synapsing with the same facial motoneurons as for the R1 response. The reflex loop of the MN-BR is less well understood (Alvarez-Blanco et al., 2009; Valls-Solé et al., 1994). Because their afferents travel via different routes (medial lemniscus in SOTN-BR and spinothalamic tract in MN-BR (León et al., 2011; Miwa et al., 1998)) SOTN-BR and MN-BR techniques are differently sensitive to differently located brainstem pathology. In upper brainstem pathology, such as in progressive supranuclear palsy or vascular lesions, MN-BR appears to be absent while SOTN-BR is present (Valls-Solé et al., 1997; León et al., 2011). The reverse pattern is observed in lower brainstem lesions (Cruccu et al., 2005; León et al., 2011). In contrast to the SOTN-BR and MN-BR the afferent signals of the ABVN-BR are conducted by the ABVN via the superior jugular ganglion to the spinal trigeminal and solitary nucleus at the dorsomedial medullary level (Nomura and Mizuno, 1984). Signals are then probably transmitted polysynaptically, along with the trigeminal nerve afferents of the SOTN-BR, to the pontomedullary RF. However, projections from the solitary nucleus to the nucleus ambiguous and dorsal nucleus of the vagus nerve and to the lateral RF are well known (Aminoff, 2005), even if they may not be part of the BR circuit.

The brainstem RF might serve as an integrator where signals are polysynaptically transmitted to the motor output neurons (Miwa et al., 1998). It is commonly accepted that connections to brainstem structures, mainly to the RF, are involved in PD pathology. Therefore, BR circuits could well be affected in PD. Furthermore, the BR may be controlled by the basal ganglia that are connected to the nuclei of the RF (Aminoff, 2005). Hence, impaired inhibitory descending control of the basal ganglia on the excitability of the brainstem in PD might also result in abnormalities in brainstem reflexes (Basso et al., 1996).

Clinically, it has been suggested that the glabella tap reflex, which might be viewed as the clinical correlate of the SOTN-BR (Kugelberg, 1952), and the palmomental reflex, possible clinical correlate of the MN-BR (Dehen et al., 1975; Gündüz et al., 2016) are disinhibited in PD and other neurodegenerative disorders (Brodsky et al., 2004) due to release from degenerating inhibitory projections to the brainstem (Pearce et al., 1968).

The auditory startle reaction, another brainstem reflex involving the RF has been found abnormal in progressive supranuclear palsy and PD, with different patterns regarding response probability, latency or amplitude (Vidailhet et al., 1992; Kofler et al., 2001). It has been proposed that a higher incidence of falls in PD might be the consequence of an impaired habituation of the startle response (Nieuwenhuijzen et al., 2006). However, in another study the MN-BR was found abnormal only in patients with progressive supranuclear palsy but not in PD patients (Valls-Solé et al., 1997). This corresponds to normal MN-BR findings in PD patients compared to healthy controls in the present study. Delayed SOTN-BR latencies have been found in patients with dementia with Lewy bodies, but not in PD patients irrespective of the presence of RBD in one study (Bonanni et al., 2007). Another study detected prolonged latencies in PD rather than in patients with multisystem atrophy and progressive supranuclear palsy. However, the mean latencies were in the normal range (Szmidt-Salkowska et al., 2016). The present study expanded the afferent routes of BR testing by introducing the ABVN-BR that involves sensory afferents conducted by the vagus nerve and the RF, where the signals are then transmitted through a polysynaptic chain of interneurons. ABVN-BR failed to unveil brainstem circuit pathology in PD. In a previous electrophysiological study employing ABVN stimulation somatosensory evoked potentials and modulation of the cutaneo-autonomic pathway as assessed by changes of the heart rate variability were normal in PD patients (Weise et al., 2015). Together, these studies may suggest that the (somato)sensory part of the vagal nerve complex and its connections to other brainstem nuclei and circuits may not be functionally affected in PD. These findings are in line with histopathological studies demonstrating that the efferent nucleus ambiguous and the afferent somatosensory nuclei are not affected by neuronal loss in PD (Del Tredici et al., 2002). However, the possibility cannot be excluded that ABVN-BR may not be sensitive enough to detect either affection of somatosensory fibres or the RF even if connections between the vagal nerve complex nuclei and the RF are substantial (Ulusoy et al., 2013; Aminoff, 2005). Up to now, affection of the vagus nerve can neurophysiologically only be indirectly assessed by measurement of heart rate variability. However, changes of heart rate variability in PD may be mainly due to sympathetic rather than parasympathetic cardiac denervation (Shibata et al., 2009). Ultrasound studies of the vagus nerve may raise another possibility to determine its pathology in vivo (Pelz et al., 2018).

One may argue that the results may have been influenced by the fact that all patients were examined in their regular ON-medication status. However, although levodopa may influence spontaneous blinking, BR kinematics do not appear to be modified by levodopa (Bologna et al., 2013). As non-motor symptoms were not explicitly assessed in this study, we cannot even indirectly infer the degree of brainstem or vagus nerve pathology, respectively, of the current study population. Furthermore, as not tested here, no conclusions can be drawn regarding habituation and prepulse variants of the BR circuit. However, the aim of the current study was to detect vagus nerve and RF pathology rather than inhibitory projections from other brain structures such as the basal ganglia that are known to be impaired in PD (Kimura, 1973; Agostino et al., 1987; Nisticò et al., 2014).

In conclusion, we here describe a novel variant of a BR involving stimulation of the ABVN. However, even if brainstem structures like the RF and nuclei of the vagal nerve complex are involved in the BR circuits and may be affected in PD we were unable to find any abnormalities in PD patients, neither with the classical trigeminal BR, nor the MN-BR, nor the ABVN-BR. We conclude that ABVN-BR testing does not constitute a promising neurophysiological technique for assessment of vagal nuclei complex functional integrity in PD. However, whether or not the present experimental approach might be relevant in distinguishing PD from atypical parkinsonism has to be proven in future studies. The findings are consistent with a highly specific pattern of brainstem pathology in PD that appears to spare reflex circuits that overlap spatially with regional pathology. Colocalization of functionally intact reflex circuits with known pathology provides indirect support for the synaptic transmission hypothesis of pathogenesis in PD (Klingelhoefer and Reichmann, 2015).

Conflict of interest

The authors declare that they have no potential conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2018.12.009.

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