Neuroendocrinology Letters Volume 37 No. 2 2016
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

Botulinum toxin treatment of freezing of gait in Parkinson's disease patients as reflected in functional magnetic resonance imaging of leg movement

Miroslav Vaštík [1], Pavel Hok [1], Petr Hluštík [1], Pavel Otruba [1], Zbyněk Tüdös [2], Petr Kaňovský [1]

1 Department of Neurology, Palacky University Medical School and University Hospital, Olomouc, Czech Republic
2 Department of Radiology, Palacky University Medical School and University Hospital, Olomouc, Czech Republic

Correspondence to: Miroslav Vaštík, MD.
Department of Neurology, Palacky University Medical School and University Hospital
I.P.Pavlova 6, Olomouc 77900, Czech Republic.
e-mail: vastik1@seznam.cz

Submitted: 2016-01-28 Accepted: 2016-02-22 Published online: 2016-04-29

Key words: **freezing; gait; Parkinson; botulinum toxin; cues; dystonia; anxiety; hippocampus; imaging; striatum; amygdala; stability**

Neuroendocrinol Lett 2016; **37**(2):147–153 **PMID:** 27179579 NEL370216A15

Abstract

BACKGROUND: Freezing of gait (FOG) is a common disabling symptom of (in) Parkinson's disease (PD). The mechanism of FOG is (in) not clearly understood. We investigated the clinical effect and changes of the activity of the sensorimotor system using repeated functional MRI (fMRI) before and after application of botulinum toxin in Parkinson's disease patients with FOG.

METHODS: We investigated 20 patients with PD, 10 with FOG and 10 without FOG. PD patients with FOG were treated with intramuscular application of botulinum toxin type A into the tensor fasciae latae muscle bilaterally. The clinical effect of treatment was assessed using FOG questionnaire, "Time up and go" test, UPDRS, Hoehn and Yahr staging, Clinical global impression scale. Activation of the sensorimotor system was studied using BOLD fMRI of the whole brain during repetitive abduction – adduction of each leg interleaved with rest. The clinical (in the FOG group) and imaging (in both groups) examination was repeated after a four-week interval.

RESULTS: In the FOG group, the FOG questionnaire has shown a decline of scores after application of botulinum toxin that suggests possible effect of botulinum toxin on freezing of gait. In fMRI results, both groups manifested reduction of the sensorimotor network activated with leg movement, however, the FOG group also showed increased activation in cerebellar vermis and nuclei, in dorsal pons and in medulla after treatment.

CONCLUSION: Alleviation of the FOG in PD patients by botulinum toxin seems to be reflected in the functional participation of the cerebellum and its projections as seen by fMRI.

ORIGINAL ARTICLE

Abbreviations:

BA	- Brodman area
BoNT	- botulinum toxin
BOLD	- blood oxygen level dependent
CNS	- central nervous system
CGSI	- clinical global impresion scale
FOG	- freezing of gait
fMRI	- functional magnetic resonance investigation
FLAIR	- fluid attenuated inversion recovery
PET	- positron emission tomography
SMA	- supplementary motor area
TUG	- time up and go
W	- week

INTRODUCTION

Freezing of gait (FOG) is an episodic disability to produce effective steps in the absence of other causes besides parkinsonism or high level gait disorders (Giladi *et al.* 2008). Types of freezing are: turning hesitation, start hesitation, tight quarters hesitation (freezing when walking through narrow spaces), destination hesitation (freezing when reaching destination). Freezing is a hallmark of idiopathic Parkinson's disease (PD), but not specific to Parkinson's disease. In fact, it is more common in other parkinsonian syndromes. It occurs more in the later stages, generally in about 40 to 60 % of patients with Parkinson´s disease. The following risk factors are considered: akinetic rigid subtype, longer duration and severity of the disease. It is more common (90%) when the effect of dopaminergic medication decreases with improvement after the use of medication, so-called "off freezing", but it may be present also in the state of good compensation – "on freezing". Freezing can appear in certain situations such as: initiation of movement, when reaching a certain destination, when upset by the time pressure to start walking (at the pedestrian crossing, the entrance to the escalator). A horizontal line, stripes on the floor, marching music, and touch stimuli can help overcome freezing and we call them cues. Patients with freezing have increased variability of walking (steps are different in length) even outside freezing episodes, so in these patients the disorders of gait rhythmicity are always present (Husdorff *et al.* 2003). For this reason, it is assumed that pathogenesis of freezing may result from lesions of structures that play a role of one or more generators of the gait pattern (Dietz, 2002). The exact pathophysiological mechanism is not known. It is assumed that during freezing, coactivation of agonistic and antagonistic muscle groups occurs (Ueno *et al.* 1993). Coactivation is classically described in dystonia, in which botulinum toxin is the treatment of choice (Giladi *et al.* 1994). Application of botulinum toxin (BoNT) to tensor fasciae latae induces response in the CNS by feedback mechanisms, central nervous system (CNS) modulation may be assessed using functional magnetic resonance imaging. Chemodenervation of the chosen muscle does not cause motor complications, which have been a problem in previous BoNT studies (Gurevich *et al.* 2007). We report the result of a pilot study which assessed the effect of BoNT using functional magnetic resonance imaging in Parkinsonism. We investigated the clinical effect and changes of the activity of the sensorimotor system using repeated functional MRI (fMRI) before and after application of botulinum toxin in Parkinson's disease patients with FOG.

MATERIAL AND METHODS

We investigated 21 patients with PD, 11 with FOG as a dominant and disabling symptom and 10 without FOG. Patient were 60 – 80 years old with disease duration of 5–15 years. Patients with FOG had average age 71.2±3.1 and patients without FOG 69.7±4.0. The groups did not differ significantly with age (unpaired Student´s t-test $p<0.05$). The inclusion criteria were: 1) probable PD defined by the presence of 3 of clinical features of: resting tremor, rigidity, bradykinesia and postural instability, with a significant sustained response to anti-PD therapy; 2) a Hohen and Yahr Stage of 3 (i.e some postural instability, physically independent) or better when "on"; 3) stable PD medication for at least 30 days; 4) FOG severe enough to need treatment; and 5) optimized on their present anti-PD therapy.

PD patients with FOG were treated with intramuscular application of botulinum toxin type A (BoNT-A) into the tensor fasciae latae muscle bilaterally. Botulinum toxin was injected under electromyography guidance, dosage 50 U per one leg. The clinical effect of treatment was assessed using FOG questionnaire, "Time up and go" test and Clinical global impression scale (CGSI). Activation of the sensorimotor system was studied using blood-oxygen-level-dependent (BOLD) fMRI of the whole brain during repetitive abduction/adduction of each leg interleaved with rest. The imaging was done in both groups at baseline (Week 0, W0), when the botulinum toxin was injected, and after 4 weeks (Week 4, W4). The clinical assessments were done at W0 visit in both groups, and were repeated at W4 in the FOG group only. Anti-PD medications were kept constant throughout the study and adverse effects were recorded at each visit. All PD medications were withheld 12 hours prior to each visit. All visits were done in the afternoon at approximately the same time.

Data acquisition

Magnetic resonance imaging data were acquired on 1.5 Tesla scanners (Avanto and Symphony, Siemens, Erlangen, Germany) with a standard head coil. Patients were scanned in supine position and were asked to have their eyes closed for the duration of the session. The MR imaging protocol covered the whole brain with 30 axial slices 5 mm thick, including fluid-attenuated inversion recovery (FLAIR) images to visualize brain lesions, functional T2*-weighted (BOLD) images during task performance and rest, and a high-resolution 3D anatomical scan (MPRAGE). BOLD images were acquired with gradient echo EPI, TR/TE = 2500/40 ms, FOV 220 mm, to provide 3.4×3.4×5 mm resolution. In total, 144 images

were acquired per each 6-minute functional run. The subject's head was immobilized with cushions to ensure maximum comfort and to minimize head movement.

During each 6-minute experimental run, patients performed self-paced repetitive abduction/adduction at hip ankle of one side. In a block design, the 15 s-long active blocks alternated with 15 s of rest, totaling 12 block pairs. Each session consisted of 4 experimental runs, two runs per leg in an interleaved order. In further analysis, only data from left leg movement were considered.

Analysis

fMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.0, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) (Jenkinson 2012). The following pre-statistics processing was applied: motion correction using MCFLIRT (Jenkinson 2002); slice-timing correction using Fourier-space time-series phase-shifting; non-brain removal using BET (Smith 2002); spatial smoothing using a Gaussian kernel of full width at half-maximum (FWHM) 10 mm; grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor; high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 15.0 s). Time-series statistical analysis was carried out using FILM with local autocorrelation correction (Woolrich 2001). To account for the subject motion and physiological noise, the volumes with severe motion were de-weighted and several nuisance signal regressors were added to the general linear model: 6 motion parameters, 6 regressors from the white-matter and 1 regressor from the ventricles (Behzadi 2007). Registration to high resolution structural and/or standard space images was carried out using FLIRT and FNIRT (Jenkinson 2001; 2002). Prior the group analysis, the within-subject repeated measures for the left leg were averaged using a fixed effects analysis (FILM).

Higher-level analysis was carried out using FLAME (FMRIB's Local Analysis of Mixed Effects) stage 1 and 2 (Beckmann 2003; Woolrich 2004). Post-hoc linear contrasts yielded Z (Gaussianized T/F) statistic images thresholded using a corrected cluster significance threshold of p=0.05 (Worsley 2001). In order to fully explore the data, several contrasts were employed: 1) group mean activation at W0 and W4; 2) between-group unpaired t-test; 3) group by time interaction (2-way repeated measures ANOVA); 4) group-wise correlations with clinical scores (FOG and TUG). In the contrast 3, the group by time interaction was additionally constrained to the areas of significant pair-wise differences in the FOG group, as the effects of the Control group were not of interest.

RESULTS

Clinical

Botulinum toxin injection was generally well tolerated, leg weakness was not noted. In the FOG group (11 patients), the FOG questionnaire has shown a decline of scores after application of botulinum toxin in 8 of the 11 patients. The overall decrease in the FOG group was 3.0 points (16.1 at W0 with standard deviation 4.68, 13.1. at W4 with standard deviation 3.62), which was significant (p<0.001, two-tailed paired t-test).

Based on CGSI: 2 patients were unchanged, 2 were markedly improved, 4 patients were moderately improved and 2 were mildly improved. The overall CGSI at W0 was 2.82 (standard deviation 0.751), this was a significant result (null hypothesis with mean CGSI at 4, p<0.005).

TUG test score has shown a decline in 6 patients, increase in 3 patients and the score was unchanged in 1 patient. The mean TUG at W0 was 36.7 (standard deviation 22.09) and decreased to 32.2 at W4 (standard deviation 15.63). The mean decrease was 4.5 (standard deviation 9.83). The difference between W0 and W4 was not significant (p=0.16).

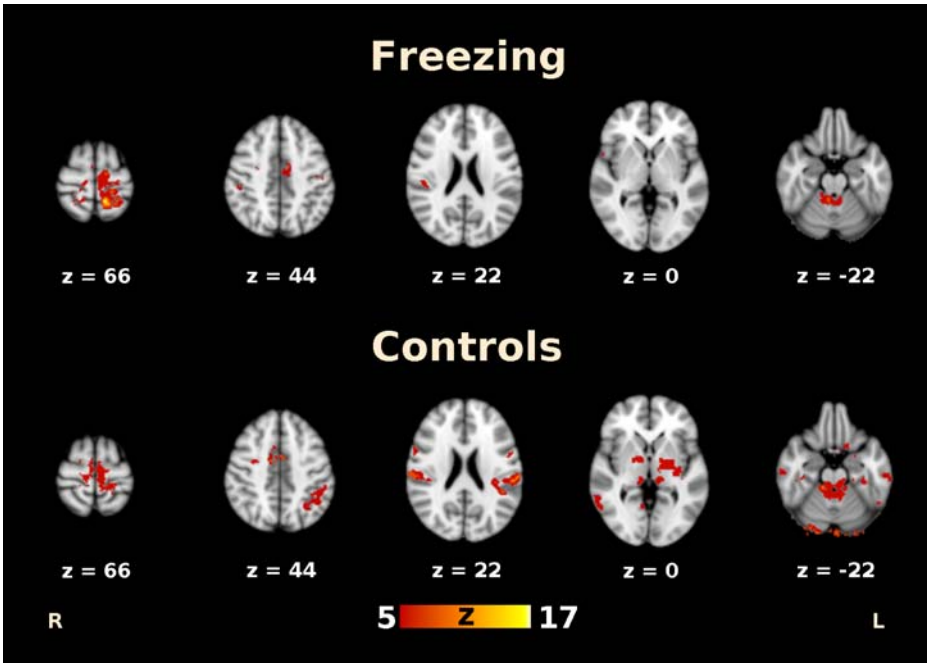

Fig. 1. Group mean functional MRI activations during leg movement. Upper row: The FOG (Freezing) group. Lower row: The control group. The red-yellow Z-statistical map was superimposed on a grey-scale mean T1-weighted anatomical image. Axial slice labels show MNI coordinates. The right of the brain is shown on the left (radiological convention).

Imaging

1) Group mean activation at W0 and W4 (Individual group mean activations during leg movement)

At baseline (W0), both groups activated a large cluster at bilateral mediodorsal primary and secondary sensorimotor cortex (precentral and postcentral gyri, Brodmann area – BA 1–6), with predominance contralaterally to the active limb, extending to the bilateral supplementary motor area (SMA, BA 6) and middle portion of cingulate gyrus (BA 24). Further clusters of activation were observed in bilateral perisylvian secondary sensory and motor cortices (BA 5,6), intraparietal sulcus bilaterally, ipsilateral frontal operculum, right fusiform gyrus, bilateral thalami, left putamen and predominantly right cerebellar hemisphere. The control group activated more the ipsilateral putamen and bilateral temporooccipital cortices. The similar activation pattern was also observed at W4 (Figure 1).

2) Between-group unpaired t-test at W0 (Baseline differences between groups)

The group comparison at W0 revealed a cluster of decreased activation in the right temporooccipital cortex (inferior division) and right occipital pole in the FOG group, as compared to the Control group (Figure 2).

3) Group by time interaction (2-way repeated measures ANOVA) in conjunction with significant pair-wise differences (Activation changes associated with BoNT therapy in the FOG group)

The whole-brain ANOVA yielded a significant interaction in the bilateral amygdalae, left hippocampus and posterior division bilateral temporal cortices, including parahippocampal, fusiform, and superior, middle and inferior temporal gyri. On the right side, the cluster extended to the right insula and right frontal orbital cortex (data not shown).

The conjunction with the respective paired contrast in the FOG Group (W0>W4) confirmed that there was a FOG-specific activation decrease in the left amygdala, hippocampus and left parahippocampal, fusiform and middle temporal gyri. Another discontinuous overlap of the original clusters was also found in the left putamen/insula (Figure 3). Since there was no significant activation increase in the Control group, this interaction was therefore driven by activation decrease in the FOG Group.

4) Covariate (FOG and TUG score) group-wise correlations (Brain networks associated with FOG severity – FOG and TUG scores)

The group-wise correlation with the FOG score showed a positive correlation in the left superior parietal lobule, left medial occipital cortex, right lateral occipital cortex

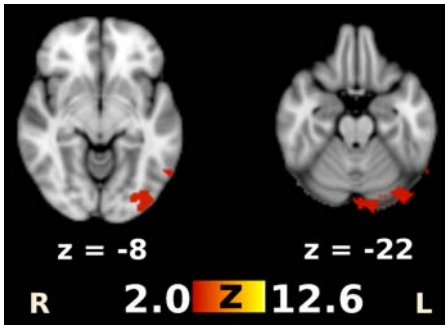

Fig. 2. Baseline differences between groups, showing clusters of decreased activation in the FOG group when compared to the Control group. Conventions see Fig. 1.

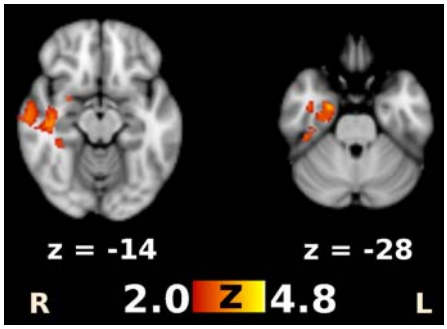

Fig. 3. Activation changes associated with BoNT therapy in the FOG group, showing post-BoNT decreases of fMRI activation in the FOG group. Conventions see Fig. 1.

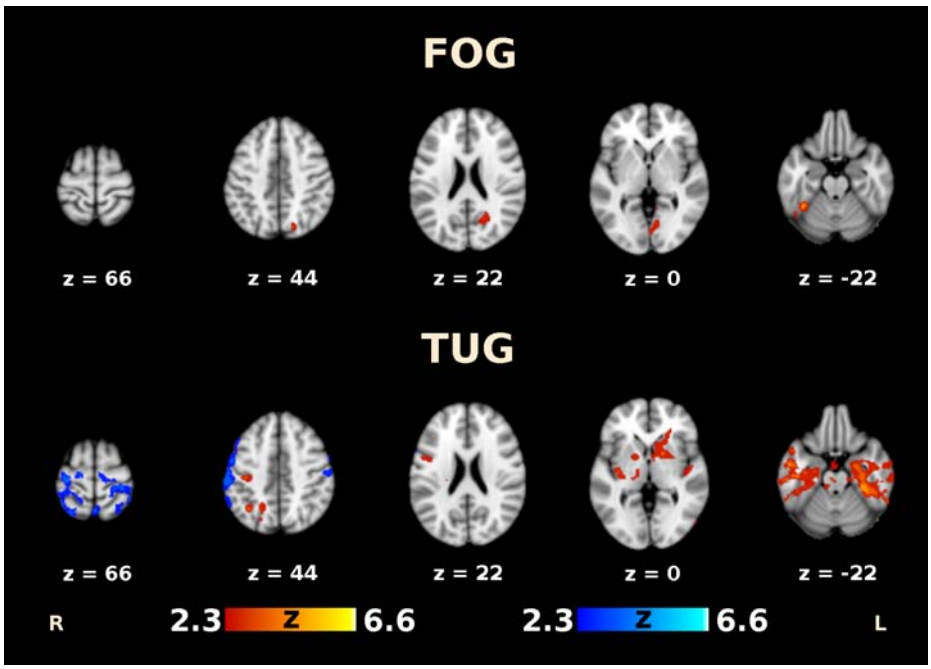

Fig. 4. Brain networks associated with FOG severity (FOG and TUG scores). Upper row: positive correlations with the FOG score. Lower row: positive (red - yellow scale) and negative (blue scale) correlations with TUG score. Remaining conventions see Fig. 1.

and right lateral cerebellum while there was no negative effect.

The group-wise correlation with the TUG score revealed a positive effect in the right superior parietal lobule, intra-parietal sulcus, medial frontal gyrus, right putamen, left caudate, bilateral temporal poles and pons, while the negative effect was observed in the mediodorsal precentral and postcentral gyrus bilaterally.

DISCUSSION

Clinical results demonstrate a significant decrease in FOG scores reflecting a possible effect of botulinum toxin application into leg muscles on the freezing of gait. Functional MRI examination during a leg motor task revealed several differences between the FOG group and the control group with PD without FOG. The BoNT treatment in the FOG group was associated with specific fMRI changes in the left temporal lobe, insula and putamen. Furthermore, there were specific brain networks associated with the clinical FOG and TUG scores, reflecting the severity of FOG in the active group. In our results, the observed baseline difference in task-related fMRI activation between the two groups may reflect abnormal visuospatial processing within occipital and temporal lobes in the FOG group. Brain activation changes associated with BoNT treatment in the FOG group of our patients manifested as decreases in several medial and lateral temporal areas. Parkinson pathology is also responsible for the reduction in amygdalar volume and the concomitant development of anxiety symptoms (Vried *et al.* 2015). It was found that FOG group reported significantly higher levels of anxiety compared to non-freezers in study of Martens and colleagues (Martens *et al.* 2014). Additionally, over 230 freezing of gait episodes were elicited (in a sample of only 14 patients with FOG) when walking in the anxious environment. This study provides strong evidence that anxiety is an important mechanism underlying freezing of gait and suggested that increasing limbic load leads to increased freezing of gait and step-to-step variability. In our study the conjunction with the respective paired contrast in the FOG Group (W0>W4) – activation changes associated with BoNT therapy in the FOG group confirmed that there was a FOG-specific activation decrease in the left amygdale. Recently, imaging studies have begun to identify neural correlates associated with freezing behavior. Although these studies did not focus on inducing anxiety to provoke freezing of gait, it is interesting that decreases in activation were found in medial prefrontal cortex, left anterior insula and left ventral striatum during motor arrest compared to walking (Shine *et al.* 2013). Although these regions are involved in an array of functions such as the cognitive control network, these areas also have a well-established role in emotional processing (Phan *et al.* 2002), and furthermore the insula has been suggested to participate in evaluation of distressing thoughts and interceptive emotional responses (Reiman *et al.* 1997). Specific activation changes were observed also in hippocampus and parahippocampal associated with BoNT therapy in the FOG group. The hippocampus has been shown to be critical for memory functioning, essential for spatial memory (Astur *et al.* 2002, 2004) what can play role in abnormal visuospatial processing. Interestingly, it has been reported that the amygdale has a direct competitive interaction with the hippocampus, so that when the amygdale activated, the hippocampus is inhibited (White *et al.* 2002). Considering the pathophysiological role of the striatum in parkinsonian motor control, finding changes in the putamen is unsurprising. Post-treatment activation changes in the putamen are certainly related to the pathophysiological role of the striatum in parkinsonian motor control. Imaging results have also shown that our FOG group has significantly less BOLD signal in the bilateral anterior insula and bilateral ventral striatum compared to non-freezers during simulated walking in virtual reality with increased cognitive load (Shine *et al.* 2013). Taken together, these results align with the current findings and theoretical framework suggesting that dysfunction processing of emotional information in the ventral striatum might be one explanation of the current results showing that anxiety increased freezing of gait. Projections from functionally different parts of the cerebral cortex to the striatum are topographically organized. The result of this organization is that the striatum can be subdivided into functionally different sectors, namely a sensorimotor sector receiving convergent inputs form motor, premotor and sensory cortical areas. An associative sector receiving inputs from (pre)frontal, temporal and parietal association cortical areas, and finally, a limbic sector that is projected upon by the hippocampus, amygdala, parahippocampal and orbitofrontal cortices (Groenewegen *et al.* 2003). The localization of changes / inclusion of medial temporal area and fusiform gyrus is likely related to spatial navigation circuits activated by gait and gait imagery, see, for example la Fougere *et al.* (2010). Even though our patients performed periodic leg movements different from those involved in gait, they also required spatial planning and it is likely that similar control areas are involved. Middle temporal gyrus activation has been previously observed in parkinsonian and not normal gait, however, its role in gait control is not clear (Hanakawa *et al.* 1999). Generally we can say that the responsible pathophysiological substrate of FOG is likely rather complex. Whereas automatic control of gait appears to be subserved by brainstem nuclei and their corresponding circuits, in FOG, involvement of cortical brain structures is presumed because of frequent manifestation of freezing in situations with emotional, voluntary or cognitive context. According to existing PET and MRI studies, freezing is a correlate of neuronal dysfunction at three main levels: 1. in

the right parietal – lateral premotor circuits that under physiological conditions convey sensory information to processes of gait control, 2. in frontostriatal circuits that process cognitive context with a participation of attention (Bartels *et al.* 2006) and 3.in corticopontine tracts that affect gait and postural stability (Herman *et al.* 2013).

LIMITATIONS

MRI methods are likely the most currently used tool in neuroscience research. Despite progress in functional MR imaging over the past 20 years, there are still technical limitations that arise from the method itself. Other limitations of fMRI result from inadequate consideration of the circuitry and functional organization of the brain, as well as from inappropriate experimental protocols that ignore this organization (Logothetis 2008). Further advances in fMRI research promise to push forward from mere cartography to the accurate study of brain organization. Functional MRI limitations in neuroscience research can be reduced by a multimodal approach, which is more necessary than ever for the study of the brain function and dysfunction. The combination of fMRI with other non-invasive techniques that directly assess the brain's electrical activity and a profound understanding of the neural basis of hemodynamic responses and animal invasive experimentation seem to be necessary (Logothetis 2008). Many factors modify the response to specific stimuli employed in the fMRI examination. For interpreting the results, it is not only the type of stimulation, which is important, but in particular also the type of instruction provided before the functional MRI investigations. In most of the studies, there is a number of general limitations related to the included patients. The common problem is still the small sample size, which places high demands on the homogeneity of the cohort. The lower number of probands may lead to the appearance of false positive differences, or vice versa can conceal truly present group differences (false negativity), even with otherwise sound methodology. Comorbid anxiety disorders, depression or personality disorders are usually present in FOG patients.

CONCLUSION

Functional brain imaging techniques appear ideally suited to explore the pathophysiology of freezing of gait (FOG). In the last two decades, techniques based on magnetic resonance or nuclear medicine imaging have found a number of structural changes and functional disconnections between subcortical and cortical regions of the locomotor network in patients with FOG. FOG seems to be related in part to disruptions in the "executive-attention" network along with regional tissue loss. Maladaptive neural compensation may present transiently in the presence of acute conflicting motor, cognitive or emotional stimulus processing, thus causing acute network overload and resulting in episodic impairment of stepping.

The present study can serve as a basis for power calculations for future controlled trials that will also investigate the not yet clearly understood clinical effect and changes of the activity of the sensorimotor system using repeated functional MRI (fMRI) before and after application of botulinum toxin in Parkinson's disease patients with FOG. Clinical results indicate that botulinum toxin injected into tensor fasciae latae can improve the manifestations of FOG. In fMRI results, application of botulinum toxin (BoNT) to tensor fasciae latae induces above mentioned response in the central nervous system (CNS) by feedback mechanisms and CNS modulation is associated with the cognitive and emotional components of FOG. The responsible pathophysiological substrate of FOG is likely rather complex, it is not clearly understood, next studies are important to investigate this mysterious phenomenon. Treatment of freezing is important because freezing of gait is a major risk factor for falls in parkinsonism and a source of patients disability. Various treatment approaches exist, including pharmacological and surgical options, as well as physiotherapy and occupational therapy, but evidence is inconclusive for many approaches, and clear treatment protocols are not available.

REFERENCES

1 Astur RS, Talylor LB *et al.* (2002). Human with hippocampus damage display sever spatial memory impairments in a virtual Morris water task. Behavioural Brain Research. **132**: 77–84.
2 Bartles AL, de Jong BM, Giladi N (2006). Striatal dopa and glucose metabolism in PD patients with freezing of gait. Mov Disord. **21**: 1326–1332.
3 Beckmann CF, Jenkinson M, Smith SM (2003). General multilevel linear modeling for group analysis in FMRI. Neuroimage. **20**: 1052–1063.
4 Behzadi Y, Restom K, Liau J, Liu TT (2007). A Component Based Noise Correction Method (CompCor) for BOLD and Perfusion Based fMRI. Neuroimage. **37**: 90–101.
5 La Fougère C, Zwergal A, Rominger A, Förster S, Fesl G, Dieterich M, Brandt T, Strupp M, Bartenstein P, Jahn K (2010). Real versus imagined locomotion: a [18F]-FDG PET-fMRI comparison. Neuroimage. **50**(4): 1589–98.
6 Giladi N, Meer J, Kidan C, Greenberg E, Gross B, Honigman S (1994). Interventional neurology: botulinum toxin as a potent symptomatic treatment in neurology. Isr J Med Sci. **30**: 816–819.
7 Groenewegen HJ (2003). The basal ganglia and motor control, Neural Plasticity. **10**(1–2): 107–120.
8 Guverich T, Peretz Ch, Moore O, Weizmann N, Giladi N (2007). The effect of injecting botulinum toxin type A into the calf muscles on freezing of gaitn in Parkinson´s disease: A double blind placebo-controlled pilot study. Mov Disord. **22**(6): 880–3.
9 Hanakawa T, Katsumi Y, Fukuyama H, Honda M, Hayashi T, Kimura J, Shibasaki H (1999). Mechanisms underlying gait disturbance in Parkinson's disease: a single photon emission computed tomography study. Brain. **122**(Pt 7): 1271–1282.
10 Herman T, Rosenberg-Katz K, Jacob Y, Giladi N, Hausdorff JM (2014). Gray matter athrophy and freezing of gait in Parkinson´s disease. Mov Disord. **29**: 134–139.
11 Jenkinson M, Beckmann CF, Behrens TEJ, Woolrich MW, Smith SM (2012). FSL. Neuroimage. **62**: 782–790.

12 Jenkinson M, Bannister P, Brady M, Smith S (2002). Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage. **17**: 825–41
13 Jenkinson M, Smith S (2001). A global optimisation method for robust affine registration of brain images. Med Image Anal. **5**(2): 143–56.
14 Logothetis NK (2008). What we can do and what we cannot do with fMRI. Nature. **453**: 869–878.
15 Lyoo CH, Aalto S, Rinne JO, Lee KO, Oh SH, Chang JW, Lee MS (2007). Different cerebral cortical areas influence the effect of subthalamic nucleus stimulation on parkinsonian motor deficits and freezing of gait. Mov Disord. **22**(15): 2176–82.
16 Martens KAE, CG Ellard, QJ Almeida (2014). Does anxiety cause freezing of gait in Parkinson's disease? PLoS ONE. **9**(9):1–10.
17 Phan KL, Wager T, Taylor SF, Liberzon I (2002). Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. Neuroimage. **16**(2): 331–48.
18 Reiman EM, Lane RD, Ahern GL, Schwartz GE, Davidson RJ, Friston KJ, Yun LS, Chen K (1997). Neuroanatomical correlates of externally and internally generated human emotion. Am J Psychiatry. **154**(7): 918–25.
19 Shine JM, Matar E, Ward PB, Frank MJ, Moustafa AA, Pearson M, Naismith SL, Lewis SJ (2013). Freezing of gait in Parkinson's disease is associated with functional decoupling between the cognitive control network and the basal ganglia. Brain. **136**(Pt 12): 3671–81.
20 Smith SM (2002). Fast robust automated brain extraction. Hum Brain Mapp. **17**: 143–55.
21 Schweder PM, Joint C, Hansen PC, Green AL, Quaghebeur G (2010).Chronic pedunculopontine nucleus stimulation restores functional connectivity. Neuroreport. **21**: 1065–1068.
22 Ueno E, Yanagisava N, Takami M (1993). Gait disorders in parkinsonism.A study with floor reaction forces and EMG. Adv Neurol. **60**: 414–418.
23 Vriend C, Boedhoe PS, Rutten S, Berendse HW, van der Werf YD, van den Heuvel OA (2015). A smaller amygdala is associated with anxiety in Parkinson's disease: a combined FreeSurfer-VBM study. J Neurol Neurosurg Psychiatry. 2015 May 18. pii: jnnp-2015-310383.
24 White NM, McDonald RJ (2002). Multiple parallel memory systems in the brain of the rat. Neurobiology of Learning and Memory. **77**: 15–184.
25 Woolrich MW, Behrens TEJ, Beckmann, CF, Jenkinson M, Smith SM (2004). Multilevel linear modelling for FMRI group analysis using Bayesian inference. NeuroImage. **21**: 1732–1747.
26 Woolrich MW, Ripley BD, Brady M, Smith SM (2001). Temporal autocorrelation in univariate linear modeling of FMRI data. Neuroimage. **14**: 1370–1386.
27 Worsley KJ (2001). Statistical analysis of activation images. In: Jezzard P, Matthews PM, Smith SM (Eds.), Functional MRI: An Introduction to Methods. Oxford University Press, Oxford [England]; New York.