Modulation of Cortical Activity in Patients Suffering from Upper Arm Spasticity following Stroke and Treated with Botulinum Toxin A: An fMRI Study

Zuzana Šenkárová, MD, Petr Hluštík, MD, MSc, PhD, Pavel Otruba, MD, Roman Herzig, MD, PhD, Petr Kaňovský, MD, PhD

From the Departments of Neurology (ZŠ, PH, PO, PH, PK) and Radiology (PH), Faculty of Medicine and Dentistry, Palacký University and University Hospital, Olomouc, Czech Republic.

ABSTRACT

BACKGROUND AND PURPOSE

Botulinum toxin (BTX) treatment can relieve focal arm spasticity after stroke, presumably through dynamic changes at multiple levels of the motor system, including the cerebral cortex. However, the neuroanatomical correlate of BTX spasticity relief is not known and should be reflected in changes of cortical activation during motor tasks assessed using repeated functional magnetic resonance imaging (fMRI).

METHODS

Four patients (2 males, 2 females, mean age 25.5 years) with hemiplegia and distal arm spasticity after chronic ischemic stroke sparing the motor cortex were studied. fMRI during mental movement simulation of the impaired hand was performed in 2 sessions before and 4 weeks after BTX treatment. The change in arm spasticity was assessed using the modified Ashworth scale (MAS).

RESULTS

BTX treatment significantly decreased arm spasticity across the group (mean MAS change 2.1). Whereas fMRI during imagined movement pre-BTX treatment showed extensive bilateral network of active areas, post-BTX activation was confined to the midline and contralateral sensorimotor cortices. The pre- > post-BTX contrast revealed a significant decrease in activation of the posterior cingulate/precuneus region after BTX treatment.

CONCLUSION

This small study suggests that structures outside the classical motor system, such as the posterior cingulate/precuneus region, may be associated with the relief of poststroke arm spasticity.

Keywords: Stroke, arm spasticity, functional magnetic resonance imaging, botulinum toxin.

Acceptance: Received July 2, 2008, and in revised form November 15, 2008. Accepted for publication February 4, 2009.

Correspondence: Address correspondence to MUDr. Zuzana Šenkárová, Department of Neurology, University Hospital, I. P. Pavlova 6, 77520 Olomouc, Czech Republic. E-mail: zsenkarova@email.cz

Conflict of Interest: There are no conflicts of interest associated with this manuscript, financial or otherwise. The manuscript has been read and approved by all authors.

The paper was presented in part as a lecture at the 16th European Stroke Conference in Glasgow, United Kingdom, in May 2007, and as a poster at the 17th Meeting of the European Neurological Society, Rhodos, Greece, in June 2007.

J Neuroimaging 2010;20:9-15.
DOI: 10.1111/j.1552-6569.2009.00375.x

Introduction

Stroke is the most important cause of neurological disabilities in adults. The clinical features and subsequent disability following stroke occur secondary to ischemia-induced neuronal loss. Damage to the corticofugal fibers (pyramidal and parapyramidal) leads to motor deficits, which are present in more than 80% of stroke patients.[1,2] These deficits, which are collectively described as the upper motor neuron syndrome, are characterized by a combination of negative phenomena (eg, muscle weakness) and positive phenomena (eg, spasticity).[2,3] Spasticity is a common impairment, which can be present in more than a half of all patients at a year following stroke. Although muscle weakness is an important factor in the motor functional disability in these patients, the contribution of muscle spasticity is often quite significant. Spasticity frequently causes problems with posture, transfers, physical therapy, nursing care, and hygiene.[4] Focal spasticity, particularly resulting from cerebral disorders, is currently being treated successfully with botulinum toxin (BTX) A injections into the spastic muscles. BTX is currently considered to be the treatment of choice in focal spasticity following stroke.[5]

BTX acts by blocking acetylcholine release at the neuromuscular junction of both extrafusal and intrafusal fibers. We hypothesize that because of this dual effect, BTX treatment can relieve focal spasticity after stroke through dynamic changes at multiple levels of the motor system, presumably including the cerebral cortex. The neuromuscular junction site of action is definite, but there is some evidence that there might be other sites of action (eg, sensory) that could contribute to the antispastic action, and there could be central nervous system (CNS) changes involved as well.[6] BTX probably alters sensory inputs to the CNS, with reduction of Ia afferent signals, and thus may indirectly induce secondary central changes. This phenomenon has been already described in dystonia and can also be presumed in cerebral spasticity.[7-9]

On the other hand, the brain hemisphere impaired by stroke is able to restore motor function of the disabled arm, probably because of the mechanism of cortical plasticity. Both these

Table 1. Patient Characteristics

Patient No.	Sex	Age (Years)	Lesion Side	Lesion Location	Hand Dominance
1	M	31	R	Thalamus, capsule, basal ganglia (BG)	N
2	M	22	L	BG, capsule	D
3	F	24	L	Thalamus, BG, capsule	D
4	F	25	R	Thalamus, capsule, BG	N
Summary	2M/2F	25.5 ± 3.4 years	2L/2R	Heterogeneous	2D/2N

L = left; R = right; D = dominant hand impaired; N = non-dominant hand impaired.

processes should be reflected in the changes of cortical activation during motor or mental tasks, as assessed using functional magnetic resonance imaging (MRI) (fMRI).

The aim of our fMRI study was to localize the changes in cerebral cortex activation in stroke patients suffering from upper limb spasticity and treated with BTX A.

Patients and Methods

Patients

The patients were recruited in the Stroke Centre at the Department of Neurology, University Hospital, Olomouc, Czech Republic, between January 2005 and January 2007. The study was conducted in accordance with the Helsinki Declaration of 1975 (as revised in 2000) and was approved by the local ethics committee of our hospital.

The study group consisted of 4 patients (2 males, 2 females; aged 25.5 ± 3.4 years, range 22-31 years). All patients suffered from distal arm plegia and spasticity of upper arm following ischemic stroke at 1+ and higher of modified Ashworth scale (MAS) and showed an ability to cooperate during the performed examinations, for example, with normal hearing and vision (corrected) and not suffering from major aphasia, and were enrolled after having provided informed consent to their participation in the study. Screening was performed to exclude patients with significant cognitive deficit (Mini-Mental State Examination [MMSE][10]), and depression (Zung self-rating depression scale[11]).

The stroke in all patients had been diagnosed by clinical history, clinical examination, and brain MR or brain computed tomography (CT) examinations. The time from stroke onset to the study entry ranged from 2 to 11 months, and the mean was 5.75 (standard deviation [SD] = 4.1 months). In all patients, the infarction was visualized by MRI at the beginning of the study to reveal its size and location.

The patients' characteristics are listed in Table 1, and the map of lesions is shown in Figure 1.

Behavioral Assessment

Spasticity was evaluated during clinical examination using the MAS.[12] The motor function was assessed quantitatively according to a dedicated physiotherapeutic protocol, which tested the functional motor skills and was developed for use in a clinical rehabilitation setting using the Gross Motor Function Measure protocol template.[13] The functional assessment was done by a rehabilitation specialist blinded to the protocol and treatment. The assessments were done at week 0, when patients were screened, enrolled, and injected with BTX, and then at week 4, 4 weeks following the injection of BTX.

Treatment

The patients were treated with BTX A injections into the muscles of the affected arm and then they underwent intensive rehabilitation treatment (according to the tailored protocol) for a period of 4 weeks. BTX A (BOTOX®; Allergan, Inc., Irvine, CA) was used for the preparation of the solution. The flexor carpi radialis, flexor carpi ulnaris, flexor digitorum superficialis, and flexor digitorum profundus muscles were always injected; the dose used for the injection into 1 muscle was always 50

Fig 1. Lesion localization in individual patients displayed on an axial Talairach-Tournoux atlas template ($z = +12$ mm). Talairach J, Tournoux P. Co-planar Stereotaxic Atlas of the Human Brain. 1st ed. Stuttgart: Georg Thieme Verlag, 1988.

U. Injection of BTX into each muscle in all 4 patients was always performed with the electromyographic (EMG) guidance, preferably with electrical stimulation for localization of the muscle intended to be treated. All patients were receiving antiplatelet therapy, so digital compression of the injection site has been performed for at least 1 minute after each injection. Fifteen minutes after injection, the injection sites were inspected.

The rehabilitation treatment was done during the admittance to the hospital ward, and the treatment was started the second day after the injection procedure. The patients underwent sessions of tailored rehabilitation treatment each day, and each such exercise session lasted 1 hour. The protocol of rehabilitation treatment was set up prior to the start of the study, and the proper adherence of rehabilitation treatment to the protocol has been repeatedly checked during the sessions.[13]

Tasks

Prior to the functional brain imaging session, the subjects practiced sequential movement of the healthy fingers (Roland's paradigm) at the rate of approximately 1 movement every second. After attaining smooth, error-free performance with the healthy hand, the subjects were asked to imagine performing the same movement with the impaired fingers. The instructions encouraged performing mental simulation of movement, associated with a kinesthetic feeling (kinetic imagery).[14]

In the MRI scanner, hand movements were performed with eyes closed; beginning- and end-of-movement blocks were signaled verbally (start/stop) in MR-compatible headphones. In a block paradigm, imagery of sequential finger movement alternated with rest (15 seconds). Each experimental run consisted of 12 repetitions of the same imagery–rest block pairs for a total of 6 minutes. Each participant had 2 experimental runs with the impaired hand. The fMRI examinations were done at week 0, when patients were screened, enrolled, and injected with BTX, and then at week 4, 4 weeks following the injection of BTX.

Data Acquisition

MRI data were acquired on 1.5-Tesla scanners (Avanto and Symphony; Siemens, Erlangen, Germany) with a standard head coil. The MR imaging protocol covered the whole brain with 30 axial slices, 5-mm thick, including anatomical T_1-weighted images to provide an immediate overlay with functional data, fluid attenuated inversion recovery (FLAIR) images to visualize brain lesions, functional T_2-weighted blood oxygen level-dependent (BOLD) images during task performance and rest, and a high-resolution 3-dimensional anatomical scan (magnetization-prepared rapid acquisition gradient echo [MPRAGE]). BOLD images were acquired with gradient echo echo planar imaging (repetition time/echo time = 2,500/40 ms, field of view = 220 mm) to provide 3.4-mm × 3.4-mm × 5.0-mm resolution. In total, 144 images were acquired per each 6-minute functional run. The subject's head was immobilized with cushions to assure maximum comfort and minimize head motion.

Analysis

Prior to fMRI analysis, the imaging data of 2 patients with right hemispheric lesion were flipped in the left–right direction to allow group analysis of movement imagery with the impaired hand.[15]

fMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool), version 5.91, part of FMRIB's software library (FSL; http://www.fmrib.ox.ac.uk/fsl). The following prestatistics processing was applied: motion correction using FMRIB's MCFLIRT[16]; slice-timing correction using Fourier space time-series phase-shifting; nonbrain removal using FMRIB's BET[17]; spatial smoothing using a Gaussian kernel of 10-mm full width at half-maximum; grand-mean intensity normalization of the entire 4-dimensional data set by a single multiplicative factor; and high-pass temporal filtering (Gaussian weighted least-squares straight-line fitting, with sigma = 15.0 seconds). Time-series statistical analysis was carried out using FMRIB's FILM with local autocorrelation correction.[18] Registration to high-resolution structural and/or standard space images was carried out using FMRIB's FLIRT.[16,19]

Higher-level analysis was carried out using FMRIB's local analysis of mixed effects (FLAME) stage 1 only (ie, without the final Metropolis-Hastings Markov Chain Monte Carlo-based stage).[20,21] Z (Gaussianized T/F) statistic images were thresholded using clusters determined by $Z > 1.5$ and a (corrected) cluster significance threshold of $P = .05$.[22]

Results

Behavioral

BTX treatment decreased arm spasticity across the group (mean MAS change 2.1, $P = .0013$, 1-sided paired t-test) measured 4 weeks following the BTX injection. The mean MAS score at week 0 was 3.5 (SD = .57), and the mean MAS score at week 4 was 1.38 points (SD = .49).

None of the patients showed any signs of clinical depression (Zung) or cognitive deficit (MMSE) during the study period.

Imaging

Group fMRI during imagined movement prior to BTX treatment showed an extensive bilateral network of active areas, including the contralateral motor cortex, supplementary motor area, bilateral premotor cortices, superior parietal lobe, precuneus/posterior cingulate, basal ganglia, and ipsilateral cerebellum (Fig 2A). fMRI activation during imagined movement after BTX treatment was limited to the midline and contralateral sensory and motor cortices (Fig 2B).

Across the patient group, the post- > pre-BTX contrast did not reveal any significant increase of cortical activation after BTX. On the other hand, the pre- > post-BTX contrast revealed a significant decrease ($P < .05$) in activation of the posterior cingulate/precuneus region after BTX treatment, a region centered on Montreal Neurological Institute coordinates (−8, −48, 0) (see Fig 3).

Discussion

Functional motor recovery after stroke commonly occurs in surviving patients in the weeks and months following the injury. There is evidence that cerebral reorganization underlies at least some of this recovery.[23] Brain plasticity means that the structure and function of the brain have a "capacity for being moulded" (Oxford English Dictionary) and implies that the brain is continually reorganizing.[23] However, in special cases,

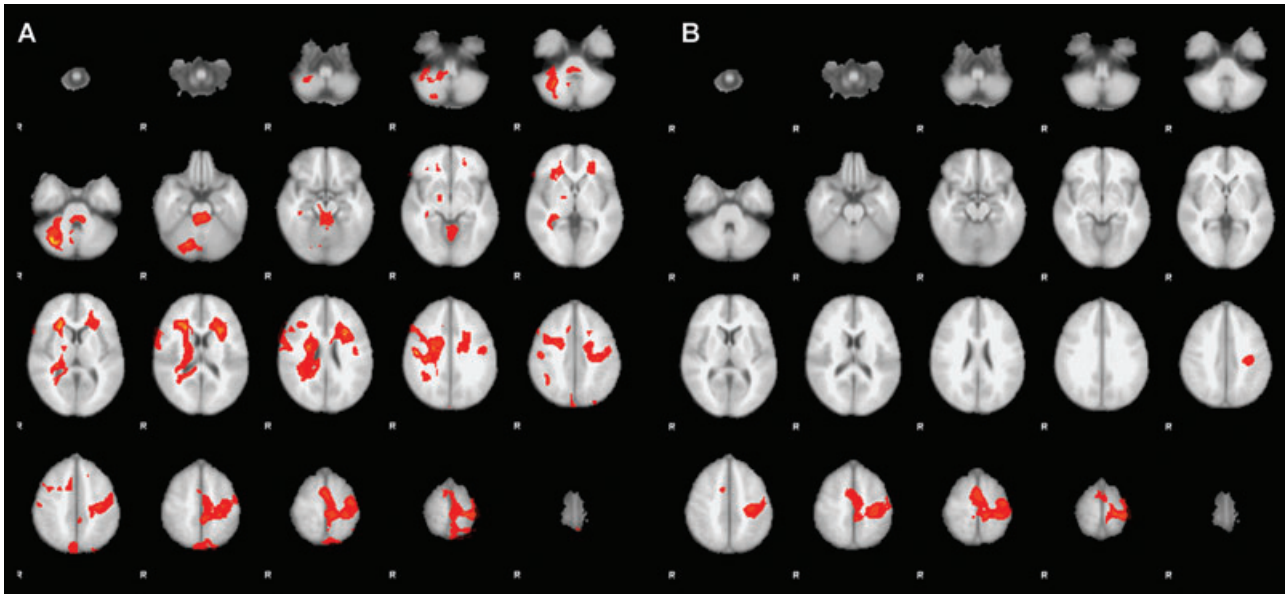

Fig 2. Functional MRI activation of the motor system during imagery of finger movement (A) before and (B) after BTX treatment of arm spasticity. Group average statistical maps (*z*-score) are overlaid in color on the MNI anatomical template. Right side of the brain is displayed on the left.

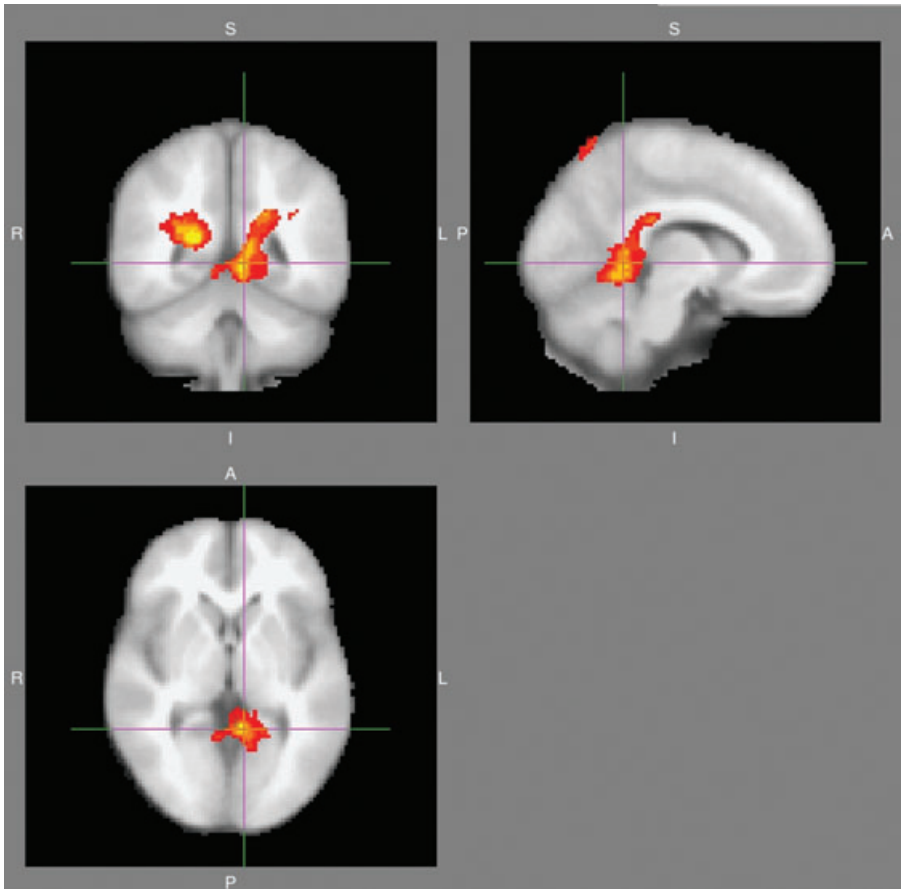

Fig 3. BTX treatment effect. Location of significant decrease in motor system activation after BTX treatment (group posthoc contrast overlaid on the MNI anatomical template). Right side of the brain is displayed on the left.

plasticity does not bring only positive benefits. Adaptations may not necessarily lead to restitution, but can even impair residual function (so-called maladaptive plasticity). Maladaptive plasticity in the brain has been reported in conditions such as dystonia, phantom limb pain after amputation, and allodynia.[24-26] The development of pain and spasticity after stroke may also be related to such maladaptive processes.

In our study of poststroke arm spasticity, we investigated the relationship between dynamic changes in movement-related brain activation and motor improvement induced by BTX treatment using fMRI. Imagery of finger movements evoked activation in the same cortical areas as those associated with performed movements[27]; the most significant regions of activation during imagery of the stroke-affected hand movement were found in the contralateral motor cortex, supplementary motor area, bilateral premotor cortices, and superior parietal lobe.

One of the observed treatment-related changes was the notable reduction in the extent and also the more prominent lateralization of the active sensorimotor network after BTX treatment (see Fig 2), similar to that shown previously.[15,28]

A novel finding was the relative decrease of activation in the posterior cingulate/precuneus region after BTX treatment, when compared with that seen in the patients prior to the treatment.

First of all, our study is limited by the small number of patients; therefore, the results should be interpreted with caution. Whether the posterior cingulate activation before the injection of BTX is due to usual functional reorganization mediated by brain plasticity following stroke, or whether this is caused by a specific condition necessary for the attempt to move a paretic hand, is not completely clear. The role of plasticity may have been more prominent in this patient group because the average patient age was low. The younger patient group emerged during our patient recruitment; the younger patients had fewer contraindications to the use of BTX.

Nevertheless, it is possible to speculate on the origins of this pretreatment activation. A potential interpretation might be that these changes represent a uniform reaction pattern of the lesioned brain as a form of maladaptation for the increased pathological proprioceptive afferentation (via Ia fibers), which is associated with spasticity.

Under physiological conditions, the cortical motor system is thought to consist of a number of independent parallel motor and sensorimotor loops that include the primary motor cortex, dorsolateral premotor cortex (PMd), supplementary motor area (SMA), cingulate motor areas (CMA), and deep gray matter structures interconnected at the cortical level and with projections to spinal cord motor neurons.[29] In the presence of spasticity, we can assume that the movement-related functional circuit is extended and includes structures outside the classical motor system.[30]

Our findings of treatment-related reduced activation of the posterior cingulate suggest that BTX probably alters impaired gating of sensory inputs to the CNS by blocking of the neuromuscular junction of the γ-motor neurons, which leads to a reduction of Ia afferent signals, and thus indirectly inhibits pre-existing feedback-driven execution mode. This hypothesis support similar previous studies investigating the central effects of BTX treatment[31,32] using techniques such as positron emission tomography and EMG that presume central reorganization following BTX treatment in patients with focal dystonia.

Although there might be some concern that spontaneous functional recovery or motor rehabilitation-related changes could affect our results, there are several reasons as to why we believe that these mechanisms do not play a role in the findings. First, the largest changes in cortical maps during recovery processes after stroke have been seen in the first few months after stroke, which is also when the steepest recovery curves are seen.[33] Our patients were all in the chronic stage of poststroke recovery; the mean period between stroke onset and the first fMRI examination in our study was 5.75 months. In the chronic stage after stroke, motor maps studied with fMRI are stable, as illustrated in the double-baseline observation of Johansen-Berg et al.[34]

Second, we compared our results with several previous studies that attempted to define the changes in brain activity that are responsible for successful recovery and rehabilitation after stroke in humans,[35-40] including 2 studies involving patients with hemiplegia.[35,36] Posterior cingulate changes were not revealed in any of these. Some of the studies also attempted to focus the investigation on regions correlated with successful motor rehabilitation,[34] but the resulting patterns did not include the posterior cingulate either; instead, other regions not present in our results (cerebellum or secondary sensory) showed changes. We therefore assume that our observed pattern of change is more likely to be related to the relief of spasticity rather than to the concomitant physical therapy.

The cingulate gyrus is a principal component of the limbic system. The anterior and posterior cingulate cortices have different cytoarchitecture and subserve distinctive functions. The anterior cingulate cortex, consisting of areas 25 and 24, primarily subserves executive functions related to the executive attention, as well as visceromotor and skeletomotor control, and responses to noxious stimuli. On the other hand, the posterior cingulate cortex, consisting of areas 29, 30, 23, and 31, has been associated with functions such as working memory, encoding of visuomotor tasks and extrapersonal space, topokinetic and topographical memory, dynamic relocation of spatial attention, and global attention.[41-43] The posterior cingulate has also been related to motor performance and motor skill.[44-47]

Although the posterior cingulate region has rarely been discussed in the context of motor recovery studies of patients, we found a decrease in activation induced by BTX treatment of poststroke spasticity. This may imply that this–classical limbic–cortex may play a role in the preparation of the movement when the classical motor structures are gated by the abnormal proprioceptive input from the spastic muscles of paretic extremity.

Conclusion

This is the first, albeit small, study suggesting that structures outside the classical motor system may be associated with the relief of poststroke arm spasticity. The posterior cingulate/precuneus region has been implicated in functions such as global attention

or complex motor learning; however, in special circumstances, it probably can modulate motor processing as well.

We thank Andrea Bártková, M.D., Martin Nevrlý, M.D., and Hana Vranová, M.D., for patient recruitment and Michal Král, M.D., for technical assistance in preparation of Figure 1. This work was supported by the IGA Ministry of Health Czech Republic grant NR8367-3/2005 and NS9920-4/2008.

References

1. Peltonen M, Stegmayr B, Asplund K. Time trends in long-term survival after stroke: the Northern Sweden Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) study, 1985–1994. *Stroke* 1998;29:1358-1365.
2. Sheean G. Neurophysiology of spasticity. In: Barnes M, Johnson G, eds. *Upper Motor Neuron Syndrome and Spasticity.* Cambridge: Cambridge University Press, 2001:12-78.
3. Burke D. Spasticity as an adaptation to pyramidal tract injury. *Adv Neurol* 1988;47:401-423.
4. Barnes MP. Medical management of spasticity in stroke. *Age Ageing* 2001;30(Suppl 1):13-16.
5. Ward AB, Aguilar M, De Beyl Z, et al. Use of botulinum toxin type A in management of adult spasticity–a European consensus statement. *J Rehabil Med* 2003;35:98-99.
6. Antonucci F, Rossi C, Gianfranceschi L, et al. Long-distance retrograde effects of botulinum neurotoxin A. *J Neurosci* 2008;28:3689-3696.
7. Kaňovský P, Bareš M, Dufek J. *Spasticita: Mechanismy, Diagnostika a Léčba.* Praha: Maxdorf, 2004.
8. Kaňovský P, Streitová H, Daniel P, et al. Long-term remission of cervical dystonia induced by treatment with botulotoxin A–signal of the possible effect on the central dystonic mechanism? (in Czech). *Ces Slov Neurol Neurochir* 1998;61:123-134.
9. Kanovsky P, Streitova H, Dufek J, et al. Change in lateralization of the P22/N30 cortical component of median nerve somatosensory evoked potentials in patients with cervical dystonia after successful treatment with botulinum toxin A. *Mov Disord* 1998;13:108-117.
10. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;12:189-198.
11. Zung WW. A self-rating depression scale. *Arch Gen Psychiatry* 1965;12:63-70.
12. Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. *Phys Ther* 1987;67:206-207.
13. Krobot A, Schusterová B, Tomsová J, et al. Specific protocol of physiotherapy in stroke patients (in Czech). *Ces Slov Neurol Neurochir* (in press).
14. Solodkin A, Hluštík P, Chen EE, et al. Fine modulation in network activation during motor execution and motor imagery. *Cereb Cortex* 2004;14:1246-1255.
15. Ward NS, Brown MM, Thompson AJ, et al. Neural correlates of motor recovery after stroke: a longitudinal fMRI study. *Brain* 2003;126:2476-2496.
16. Jenkinson M, Bannister P, Brady M, et al. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage* 2002;17:825-841.
17. Smith SM. Fast robust automated brain extraction. *Hum Brain Mapp* 2002;17:143-155.
18. Woolrich MW, Ripley BD, Brady M, et al. Temporal autocorrelation in univariate linear modeling of FMRI data. *Neuroimage* 2001;14:1370-1386.
19. Jenkinson M, Smith S. A global optimisation method for robust affine registration of brain images. *Med Image Anal* 2001;5:143-156.
20. Beckmann CF, Jenkinson M, Smith SM. General multilevel linear modeling for group analysis in FMRI. *Neuroimage* 2003;20:1052-1063.
21. Woolrich MW, Behrens TE, Beckmann CF, et al. Multilevel linear modelling for FMRI group analysis using Bayesian inference. *Neuroimage* 2004;21:1732-1747.
22. Worsley KJ. Statistical analysis of activation images. In: Jezzard P, Matthews PM, Smith SM, eds. *Functional MRI: an Introduction to Methods*. Oxford: Oxford University Press, 2001:251-270.
23. Pascual-Leone A, Amedi A, Fregni F, et al. The plastic human brain cortex. *Annu Rev Neurosci* 2005;28:377-401.
24. Pujol J, Roset-Llobet J, Rosines-Cubells D, et al. Brain cortical activation during guitar-induced hand dystonia studied by functional MRI. *Neuroimage* 2000;12:257-267.
25. Flor H. Remapping somatosensory cortex after injury. *Adv Neurol* 2003;93:195-204.
26. Maihofner C, Handwerker HO, Birklein F. Functional imaging of allodynia in complex regional pain syndrome. *Neurology* 2006;66:711-717.
27. Roth M, Decety J, Raybaudi M, et al. Possible involvement of primary motor cortex in mentally simulated movement: a functional magnetic resonance imaging study. *Neuroreport* 1996;7:1280-1284.
28. Ward NS, Brown MM, Thompson AJ, et al. Neural correlates of outcome after stroke: a cross-sectional fMRI study. *Brain* 2003;126:1430-1448.
29. Strick PL. Anatomical organization of multiple motor areas in the frontal lobe: implications for recovery of function. [Review]. *Adv Neurol* 1987;47:293-312.
30. Weiller C, Chollet F, Friston KJ, et al. Functional reorganization of the brain in recovery from striatocapsular infarction in man. *Ann Neurol* 1992;31:463-472.
31. Gelb DJ, Yoshimura DM, Olney RK, et al. Change in pattern of muscle activity following botulinum toxin injections for torticollis. *Ann Neurol* 1991;29:370-376.
32. Ceballos-Baumann AO, Sheean G, Passingham RE, et al. Botulinum toxin does not reverse the cortical dysfunction associated with writer's cramp. A PET study. *Brain* 1997;120(Pt 4):571-582.
33. Traversa R, Cicinelli P, Oliveri M, et al. Neurophysiological follow-up of motor cortical output in stroke patients. *Clin Neurophysiol* 2000;111:1695-1703.
34. Johansen-Berg H, Dawes H, Guy C, et al. Correlation between motor improvements and altered fMRI activity after rehabilitative therapy. *Brain* 2002;125:2731-2742.
35. Nelles G, Spiekramann G, Jueptner M, et al. Evolution of functional reorganization in hemiplegic stroke: a serial positron emission tomographic activation study. *Ann Neurol* 1999;46:901-909.
36. Marshall RS, Perera GM, Lazar RM, et al. Evolution of cortical activation during recovery from corticospinal tract infarction. *Stroke* 2000;31:656-661.
37. Liepert J, Bauder H, Wolfgang HR, et al. Treatment-induced cortical reorganization after stroke in humans. *Stroke* 2000;31:1210-1216.
38. Liepert J, Uhde I, Graf S, et al. Motor cortex plasticity during forced-use therapy in stroke patients: a preliminary study. *J Neurol* 2001;248:315-321.
39. Nelles G, Jentzen W, Jueptner M, et al. Arm training induced brain plasticity in stroke studied with serial positron emission tomography. *Neuroimage* 2001;13:1146-1154.
40. Small SL, Hluštík P, Noll DC, et al. Cerebellar hemispheric activation ipsilateral to the paretic hand correlates with functional recovery after stroke. *Brain* 2002;125:1544-1557.
41. Vogt BA, Absher JR, Bush G. Human retrosplenial cortex: where is it and is it involved in emotion? *Trends Neurosci* 2000;23:195-197.

42. Mesulam MM, Nobre AC, Kim YH, et al. Heterogeneity of cingulate contributions to spatial attention. *Neuroimage* 2001;13:1065-1072.
43. Raichle ME, MacLeod AM, Snyder AZ, et al. A default mode of brain function. *Proc Natl Acad Sci U S A* 2001;98:676-682.
44. Jancke L, Shah NJ, Peters M. Cortical activations in primary and secondary motor areas for complex bimanual movements in professional pianists. *Cogn Brain Res* 2000;10:177-183.
45. Puttemans V, Wenderoth N, Swinnen SP. Changes in brain activation during the acquisition of a multifrequency bimanual coordination task: from the cognitive stage to advanced levels of automaticity. *J Neurosci* 2005;25:4270-4278.
46. Sakai K, Hikosaka O, Miyauchi S, et al. Transition of brain activation from frontal to parietal areas in visuomotor sequence learning. *J Neurosci* 1998;18:1827-1840.
47. Milton J, Solodkin A, Hlustik P, et al. The mind of expert motor performance is cool and focused. *Neuroimage* 2007;35:804-813.