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Increase in prefrontal cortical volume following cognitive behavioural therapy in patients with chronic fatigue syndrome

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Chronic fatigue syndrome (CFS) is a disabling disorder, characterized by persistent or relapsing fatigue. Recent studies have detected a decrease in cortical grey matter volume in patients with CFS, but it is unclear whether this cerebral atrophy constitutes a cause or a consequence of the disease. Cognitive behavioural therapy (CBT) is an effective behavioural intervention for CFS, which combines a rehabilitative approach of a graded increase in physical activity with a psychological approach that addresses thoughts and beliefs about CFS which may impair recovery. Here, we test the hypothesis that cerebral atrophy may be a reversible state that can ameliorate with successful CBT. We have quantified cerebral structural changes in 22 CFS patients that underwent CBT and 22 healthy control participants. At baseline, CFS patients had significantly lower grey matter volume than healthy control participants. CBT intervention led to a significant improvement in health status, physical activity and cognitive performance. Crucially, CFS patients showed a significant increase in grey matter volume, localized in the lateral prefrontal cortex. This change in cerebral volume was related to improvements in cognitive speed in the CFS patients. Our findings indicate that the cerebral atrophy associated with CFS is partially reversed after effective CBT. This result provides an example of macroscopic cortical plasticity in the adult human brain, demonstrating a surprisingly dynamic relation between behavioural state and cerebral anatomy. Furthermore, our results reveal a possible neurobiological substrate of psychotherapeutic treatment.

Keywords: neural plasticity; prefrontal cortex; grey matter increase; cognitive behavioural therapy

Abbreviations: CFS = chronic fatigue syndrome; HC = healthy controls; CBT = cognitive behavioural therapy; VBM = voxel-based morphometry; MRI = magnetic resonance imaging; GMV = grey matter volume; SRT = simple reaction time task; CRT = choice reaction time task; WAIS-dst = digit symbol subtest of the Wechsler Adult Intelligence Scale

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Introduction

The chronic fatigue syndrome (CFS) is an illness characterized by profound disabling fatigue, of new or definite onset and lasting at least 6 months. CFS substantially limits occupational, educational, social and personal activities (Fukuda *et al.*, 1994). Infectious, immunological, neuroendocrine, sleep and psychological mechanisms have been proposed to play a role, but the exact pathogenesis has still to be elucidated (Prins *et al.*, 2006). Several symptoms reported by CFS patients—including fatigue; impaired concentration, attention and memory; and headache—suggest that the central nervous system may be involved in the pathophysiology of the syndrome. Several studies have reported structural abnormalities in the central nervous system of CFS patients, such as focal white matter hyperintensities (Buchwald *et al.*, 1992; Natelson *et al.*, 1993; Schwartz *et al.*, 1994; Lange *et al.*, 1999), although other groups failed to replicate this finding (Cope *et al.*, 1995; Cope and David, 1996). In two recent studies, we and others, using an automated procedure for assessing cerebral abnormalities, observed marked reductions in grey matter volume (de Lange *et al.*, 2005), localized to the lateral prefrontal cortex (Okada *et al.*, 2004). Since grey matter

volume reduction and white matter hyperintensities appear to be tightly linked manifestations of the same pathophysiological process (Wen *et al.*, 2006), together these results provide converging evidence that CFS is associated with brain atrophy. However, it remains unclear whether these alterations are aetiological factors or a consequence of the disease. One way to address this issue is to examine whether these cerebral structural alterations change over the course of the disease.

Structural neuroplasticity—the brain's ability to undergo structural alterations following environmental changes—has become an accepted biological phenomenon, even in the brain of adult primates (Kozorovitskiy *et al.*, 2005). These structural alterations range from biochemical parameters to dendritic arborization, gliogenesis and perhaps even neurogenesis (Buonomano and Merzenich, 1998; Gross, 2000; van Praag *et al.*, 2000). Environmental impoverishment evokes a down-regulation of cerebral structure, whereas environmental enrichment can generate an up-regulation of neuroplasticity (Rosenzweig and Bennett, 1996).

In this perspective, it is possible that the reduced grey matter volume observed in CFS is an expression of neuronal down-regulation caused by the environmental impoverishment associated with CFS. Therefore, it is also conceivable that improvements in health status of CFS patients could lead to an up-regulation of this process. We test these hypotheses by quantifying behavioural parameters and cerebral structure in a sample of CFS patients both before and after a well-known behavioural therapeutic intervention: cognitive behavioural therapy.

Cognitive behavioural therapy (CBT) and graded exercise programs have been shown to improve the health status of CFS patients (Prins *et al.*, 2001; Whiting *et al.*, 2001; Edmonds *et al.*, 2004; Stulemeijer *et al.*, 2005). CBT combines a rehabilitative approach of a graded increase in activity with a psychological approach which addresses thoughts and beliefs about CFS which may impair recovery. It is aimed at reducing perpetuating factors of CFS, like low physical activity and a low sense of control over the symptoms (Bleijenberg *et al.*, 2003*b*). Central CBT components for CFS include explanation of the aetiological model, motivation for CBT, challenging and changing of fatigue-related cognitions, achievement and maintenance of a basic amount of physical activity, gradual increase in physical activity, and planning work rehabilitation or rehabilitation in other personal activities (Prins *et al.*, 2006).

In this study we quantified cerebral structure and behavioural parameters (physical activity, cognitive performance and experienced fatigue) in a sample of CFS patients both before and after CBT. We hypothesized that CFS patients would show a reduction in grey matter volume compared to healthy controls before CBT, and an improvement in grey matter volume following CBT. Moreover, we hypothesized that this cerebral change would be related to the behavioural improvement.

Methods

Participants

We restricted our investigation to female participants, since CFS predominantly affects women, and in view of the differences in brain morphology and size between men and women (Gur *et al.*, 1991). Twenty-nine female patients who were diagnosed with CFS were willing to take part in the study. Of these, four patients withdrew from CBT, one patient was not willing to participate in the follow-up scan, one patient had to be excluded due to poor quality of the MR image, and one patient manifested somatic comorbidity during treatment. The remaining 22 patients conformed to the US Centers for Disease Control and Prevention (CDC) criteria for CFS (Fukuda *et al.*, 1994). We included 22 healthy control subjects that were matched on sex, age and education. None of the participants took any drugs that acted on the central nervous system. Demographic characteristics of the final set of participants are listed in Table 1.

All participants took part in the study after giving written informed consent according to institutional guidelines of the local ethics committee and in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Multidimensional assessments and structural brain images were made at baseline (i.e. before CBT) and at follow-up (i.e. after CBT) in all CFS patients. Healthy control participants were assessed at a comparable time interval.

Cognitive behavioural therapy

Participants engaged in cognitive behavioural therapy for on average 16 sessions of 1 h over a period of 6–9 months. During CBT, fatigue-related cognitions were challenged to diminish somatic attributions, to improve sense of control over symptoms and to facilitate behavioural changes. In parallel, a structured physical activity program was implemented. Furthermore, a work rehabilitation schedule was drawn up in order to realize a gradual work reentry. Final sessions of CBT dealt with relapse prevention and further improvement of self-control. This procedure is described in detail elsewhere (Bleijenberg *et al.*, 2003*a*).

Behavioural and psychological assessment

We collected several behavioural and psychological measures before and after CBT in all CFS patients to quantify both objective and subjective markers of fatigue and physical and mental inability.

The physical activity level of the CFS patients was assessed by actometer measurements for a period of 2 weeks preceding the baseline scan, and for a period of 2 weeks preceding the follow-up scan. The actometer (Actilog V3.0) is a motion sensing device, worn

Table 1 Demographic characteristics of participants

	CFS	HC	*P*
Male/Female participants	0/22	0/22	
Age in years	36.6 (2.5)	37.1 (2.2)	0.88
Educational attainment (1=low to 7=high)	4.7 (0.30)	4.6 (0.49)	0.92
Time between baseline and follow-up in days	312 (17)	296 (20)	0.56
CFS duration in years	5.8 (0.79)	0 (0)	<0.001

Values are given as means (SE).

at the ankle, which can register and quantify human physical activity. The average physical activity score reflects the average physical activity level over a total 12-day period (the first and last day of registration were omitted to obtain 12 complete registration days) and it is expressed as the mean number of accelerations per 5-min period (Vercoulen *et al.*, 1997). On the basis of the average physical activity score, we distinguished between pervasively passive and relatively active CFS patients (van der Werf *et al.*, 2000).

To assess cognitive speed, we used two speeded reaction time tasks: the digit symbol substitution test of the Wechsler Adult Intelligence Scale (WAIS-dst) (Wechsler, 1981) and the choice reaction time task (CRT) (Vercoulen *et al.*, 1998). We also used the simple reaction time task (SRT) to control for sensorimotor speed. In the WAIS-dst, a series of symbols is presented that has to be decoded as fast as possible within a preset 90 s limit based on a key translating the nine different symbols into the digits 1–9. In both the CRT and SRT tasks, subjects have to move as quickly as possible to a target light when it is flashed. In the SRT, only one target was used, whereas in the CRT three different targets were used. Therefore, although both tasks challenge subjects' speed of sensorimotor processing, CRT places additional demands on response selection. Each patient engaged in 30 trials of SRT and CRT.

Perceived fatigue severity was assessed by a subscale of the checklist individual strength (Vercoulen *et al.*, 1994; Dittner *et al.*, 2004). In this questionnaire, the patient is asked about fatigue in the 2 weeks preceding the assessment. The subscale consists of eight items, each scored on a 7-point Likert scale (range 8–56). The questionnaire has good reliability (Cronbach's α varying from 0.83 to 0.92) and discriminative validity (Vercoulen *et al.*, 1994). Finally, perceived disability was measured by the sickness impact profile (Bergner *et al.*, 1981). This widely used measure has good reliability and content validity (de Bruin *et al.*, 1992).

Imaging protocol

High-resolution anatomical images of the whole brain were acquired on a 1.5 T Siemens Sonata whole-body scanner (Erlangen, Germany) using a magnetization prepared rapid acquisition gradient echo sequence, acquiring 176 sagittal slices with a echo time (TE) of 3.93 ms, a repetition time (TR) of 2250 ms, voxel size of 1 mm^3, flip angle (FA) of 15° and a field-of-view (FOV) of 256 mm. This sequence gives excellent grey/white matter segmentation, and high contrast-to-noise images of the entire brain at isotropic 1 mm resolution (Brant-Zawadzki *et al.*, 1992; Deichmann *et al.*, 2000). Images were analysed using VBM (Ashburner and Friston, 2000), a fully automated technique for computational analysis of differences in global and local grey and/or white matter volume. VBM is a set of procedures within the framework of SPM (www.fil.ion.ucl.ac.uk/spm). The reliability of VBM for quantifying brain tissue volume has been extensively validated (Good *et al.*, 2001; Chard *et al.*, 2002). We first created a custom template and priors with reduced population bias, on the basis of both baseline and follow-up MR scans. This procedure entailed affine spatial normalization of all scans to the International Consortium for Brain Mapping template (Montreal Neurological Institute, Montreal, Canada), which approximates Talairach and Tournoux space (Talairach and Tournoux, 1988). The normalized images of all participants (both CFS patients and HC) were averaged and smoothed with a Gaussian kernel of 8 mm full-width at half-maximum. This procedure is described in detail by (Good *et al.*, 2001). Global (differences) in grey matter volume and white matter volume were quantified by integrating the tissue probabilities over all voxels. In order to avoid possible edge effects around the border between grey and white matter, we used an absolute grey and white matter threshold of $P < 0.15$.

Assessment of both regional and global differences in grey matter volume (GMV) between groups was preceded by the following pre-processing steps: (i) spatial normalization of all images to a standardized anatomical space by removing differences in overall size, position and global shape; (ii) extraction of grey and white matter from the normalized images; (iii) correction for volume changes induced by normalization (i.e. modulation) and (iv) smoothing with a Gaussian kernel of 12 mm full-width at half-maximum. Assessment of longitudinal changes in regional GM involved the following steps: (i) coregistration of baseline and follow-up images for each participant; (ii) spatial normalization of baseline and follow-up image to a standardized anatomical space, on the basis of the estimated normalization parameters for the baseline image; (iii) extraction of grey and white matter from the normalized images; (iv) calculation of the difference images by subtracting the baseline GMV image from the follow-up GMV image and (v) spatially smoothing the difference images with a Gaussian kernel of 12 mm full-width at half-maximum. This protocol has been successfully applied in a previous longitudinal study (Draganski *et al.*, 2004).

Statistical analysis

Global differences in grey and white matter volume between groups were assessed using a two-tailed multivariate linear regression analysis that considered age as a covariate.

Regional (i.e. voxel-by-voxel) differences in grey matter volume between groups were assessed with *t*-tests using the general linear model, considering age and total grey matter volume as confounding covariates, and correcting the results for search volume by using family-wise error (FWE) correction. We used small volume correction for investigation of regional effects in prefrontal cortex, on the basis of a previous study showing reduced GM in CFS in bilateral prefrontal cortex (Okada *et al.*, 2004). We defined regions of interest (ROIs) with a radius equal to the smoothing kernel of our images around the peak coordinates of that study. All reported regional effects survived correction for search volume using both FWE (Worsley *et al.*, 1996) and FDR (Genovese *et al.*, 2002) correction for multiple comparisons. To investigate whether CBT led to a differential increase in global grey matter between groups, we used a one-tailed multivariate linear regression analysis that considered age as a covariate. Relations between (changes in) CFS-related features and (changes in) GMV were assessed by means of one-tailed Spearman's correlation analyses. Significance level was set at $P < 0.05$ in all analyses. We screened for potential outliers and their influence on the correlation analyses. In order to identify univariate outliers, we *Z*-transformed our dependent variable of interest and employed an absolute *Z*-score of $|Z| > 2.5$ as an outlier criterion. We also checked for multivariate outliers using Mahalanobis D^2 distance measure, considering an observation an outlier if the probability associated with its D^2 is 0.001 or less.

Results

We measured global and local GMV in CFS patients and matched healthy control subjects at two time-points: baseline

(preceding cognitive behavioural therapy) and follow-up (after the CFS patients had undergone CBT treatment).

Baseline

CFS patients had significantly lower GMV than controls at baseline [$t(2,41) = 2.55$; $P = 0.015$], showing an average reduction of 5.5% in GMV compared to controls (Fig. 1A). There were no significant differences between groups in global white matter [$t(2,41) = 1.32$; $P = 0.194$]. GMV decreased with age in both groups [$t(2,41) = –4.27$; $P < 0.001$], at an average rate of 3.1 ml/year (Fig. 1B). An analysis of regional volume differences between groups did not show any significant differences in local grey matter between groups that survived correction for multiple comparisons over the whole brain.

We collected several behavioural and psychological measures before and after CBT in all CFS patients to quantify both objective and subjective markers of fatigue and physical and mental inability. We measured cognitive speed with two speeded tasks: the digit symbol subtest of the WAIS (Wechsler, 1981) and the CRT (Vercoulen *et al.*, 1998).

As illustrated in Fig. 4A and B, both measures of cognitive speed bore relation to baseline GMV in the CFS patients. CFS patients who could complete fewer items on the WAIS-dst exhibited lower GMV ($r = 0.64$, $P = 0.001$; Fig. 4A). Similarly, CFS patients who were slower at the CRT exhibited lower GMV ($r = –0.40$, $P = 0.033$; Fig. 4C). Importantly, behavioural performance on the SRT, which measures sensorimotor rather than cognitive speed, showed no significant correlation with GMV ($r = –0.15$, $P = 0.26$).

The physical activity level of the CFS patients was assessed by actometer measurements for a period of 2 weeks preceding the baseline scan, and for a period of 2 weeks preceding the follow-up scan. The activity scores and patterns of the CFS patients are listed in Table 1. Although there was no significant linear relationship between the mean physical activity scores and GMV ($r = 0.23$; $P = 0.15$), the overall physical activity pattern predicted GMV within the CFS patient group: passive CFS patients had lower GMV than active CFS patients [$t_{(20)} = –2.17$; $P = 0.042$]. Note that passive and active CFS patients did not significantly differ from each other in terms of age, degree of functional impairments or perceived fatigue.

We also collected subjective reports on perceived fatigue and disability. CFS patients reported more functional impairments and more fatigue than healthy controls (Table 2), but there were no significant correlations between GMV and these subjective reports (all $P > 0.10$).

Follow-up

Within the healthy group, GMV did not change significantly between the first and second measurement [difference: $–0.21 \pm 1.7$ ml, mean $\pm$ SE; $t_{(21)} = –0.13$; $P = 0.45$; Fig. 2A]. Conversely, there was an increase in GMV in the CFS group [difference: 4.7 ± 2.3 ml, mean $\pm$ SE; $t_{(21)} = 2.08$; $P = 0.025$; Fig. 2A], such that the initial between-group difference in GMV decreased by 12% following CBT. This between-groups difference in GMV change resulted in a significant Group X Time interaction [$t(2,41) = 1.82$; $P = 0.038$]. Patient's age also influenced the increase in GMV: the potential for an increase in GMV linearly decreased with age ($r = –0.45$; $P = 0.018$; Fig. 2B). In spite of the increase in GMV in the CFS patients after CBT, GMV was still lower in the CFS group than in the HC group [$t(2,41) = 2.21$; $P = 0.033$]. Overall, the test–retest reliability of the GMV measurements was high, both in the healthy control group ($r = 0.991$; $P < 0.001$) and the CFS patients ($r = 0.990$; $P < 0.001$).

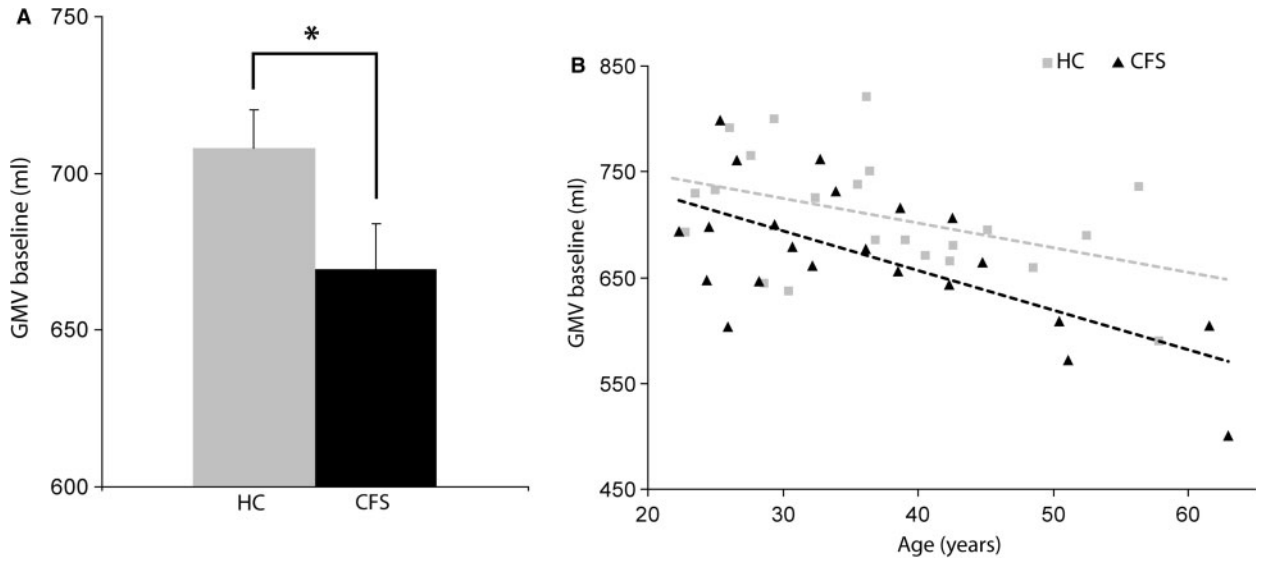

Fig. 1 Grey matter volume at baseline measurement. (**A**) Healthy controls (HC, in grey) have significantly larger grey matter volume (GMV) than CFS patients (CFS, in black). (**B**) Both groups show a similar decline in GMV as a function of ageing.

Within-group analysis of regional changes in grey matter volume between the pre- and post-CBT scans revealed significant regional increases bilaterally in the inferior frontal gyrus [left IFG: (–36, 39, 17), $t_{(21)} = 5.49$, $P_{FWE} = 0.010$, $P_{FDR} = 0.025$; right IFG: (39, 35, 23), $t_{(21)} = 5.49$, $P_{FWE} = 0.005$, $P_{FDR} = 0.011$] for the CFS group (Fig. 3), but no changes for the healthy controls. Both these clusters were located at the most dorsal portion of the inferior frontal gyrus and in the depth of the middle frontal sulcus, within the borders of cyto-architectonically defined Brodmann area 46/9 (Rajkowska and Goldman-Rakic, 1995).

In terms of cognitive speed, CFS patients became faster on the CRT ($t_{21} = 2.30$, $P = 0.032$), while they did not show

Table 2 Baseline and follow-up physical and cognitive performance of participants

	Baseline	Follow-up	Difference
CFS patients			
Functional status			
CIS fatigue (8–56)[a]	48.4(1.3)	29.3 (2.9)	−19.0 (2.9)***
SIP total (0–9937)[a]	1334 (124)	550 (115)	−784 (128)***
Physical activity			
Mean actometer score	63.5 (4.6)	73.2 (3.5)	+9.7 (4.8)*
Pervasively passive (%)	32	9	−23
Relatively active (%)	68	91	+23
Cognitive speed			
WAIS-dst	60.2 (2.8)	62.5 (2.9)	2.3 (1.6)NS
CRT (ms)	348 (14.2)	329 (11.5)	−19.4 (8.4)**
SRT (ms)	307 (11.6)	297 (12.8)	−10.3 (9.6)NS
Cerebral volume			
Grey matter (ml)	669.4 (14.4)	674.1 (15.1)	+4.7 (2.3)**
White matter (ml)	406.1 (8.9)	404.7 (8.9)	−1.4 (1.0)NS
Healthy controls			
Functional status			
CIS fatigue (8–56)[a]	21.2 (2.4)	18.7 (2.1)	−2.5 (2.7)NS
SIP total (0–9937)[a]	131 (63)	169 (69)	+38 (36)NS
Cerebral volume			
Grey matter (ml)	708.2 (12.0)	708.0 (12.6)	−0.2 (1.7)NS
White matter (ml)	423.2 (9.0)	421.4 (8.7)	−1.8 (1.7)NS

Values are given as means (SE).
[a]Range of the scale.NS = Not significant. *$p < 0.1$. **$p < 0.05$. ***$p < 0.001$.

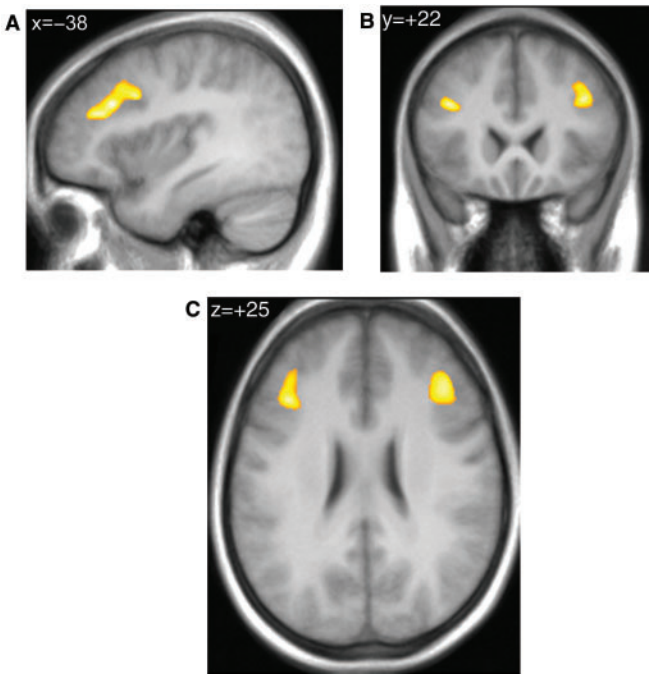

Fig. 3 Anatomical localization of grey matter volume increase. Results are superimposed on the average normalized brain of all CFS patients. There is a significant increase in prefrontal grey matter volume (BA 46/9) as a result of CBT in CFS patients. (**A**) sagittal view. (**B**) Coronal view. (**C**) Axial view. Results are displayed at $P < 0.001$ uncorrected, for display purposes.

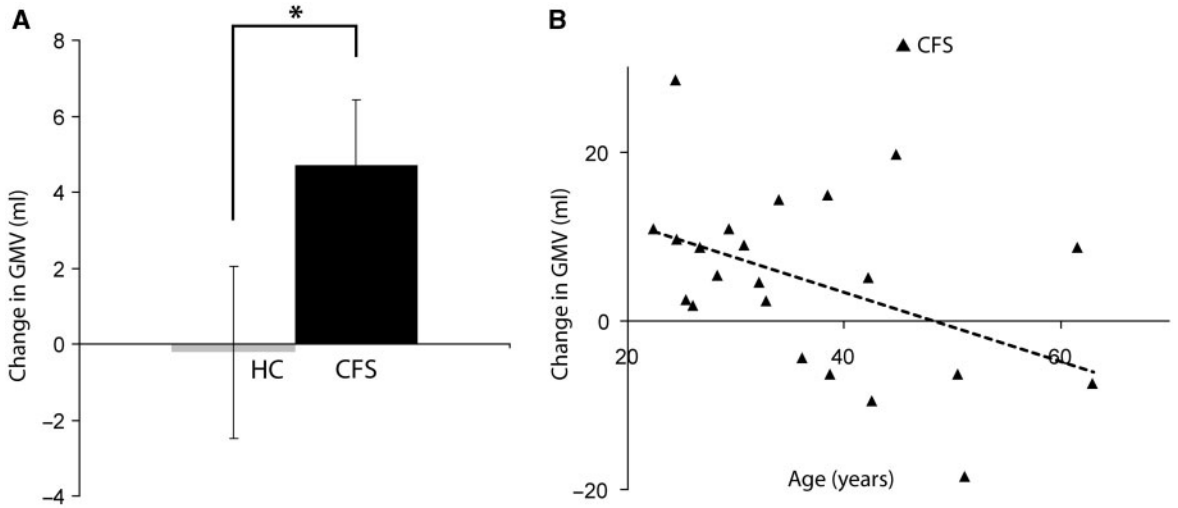

Fig. 2 Effect of CBT on grey matter volume. (**A**) There is a significant increase in GMV between baseline and follow-up in CFS patients, but not in healthy control subjects. (**B**) There is a negative correlation between the increase in GMV following CBT and the patient's age.

a significant improvement on the WAIS-dst ($t_{21} = 1.40$, $P = 0.18$). Importantly, the change in cognitive speed was correlated with the change in GMV (WAIS-dst: $r = 0.41$, $P = 0.028$; CRT: $r = 0.42$, $P = 0.027$; Fig. 4C and D). Thus, a larger behavioural improvement in cognitive speed was related to a larger increase in GMV following CBT.

There were correlations between local grey matter increase and CRT [left PFC: (–38, 39, 17), $T = 2.15$, $r = 0.43$, $P = 0.022$, $k = 113$ voxels; right PFC: (22, 47, 36), $T = 2.46$, $r = 0.48$, $P = 0.011$, $k = 691$ voxels], and between local grey matter increase and WAIS-dst in the right PFC [(26,38,49), $T = 3.45$, $r = 0.61$, $P = 0.001$, $k = 1636$]. These results were obtained by focusing the search within the prefrontal cortex, and they should therefore be considered as *post hoc* confirmatory analyses.

Patients' sensorimotor speed, as indexed by the SRT, did not change ($t_{21} = 1.08$, $P = 0.30$), and there was no correlation between SRT change and the GMV increase ($r = 0.24$, $P = 0.14$).

There was a trend of CFS patients becoming more physically active following CBT [$t_{(21)} = 2.02$, $P = 0.056$]. This increase in physical activity level was marginally correlated with the increase in GMV ($r = 0.30$; $P = 0.086$).

Following CBT, CFS patients reported less functional impairments (SIP-total) and experienced less fatigue (CIS-fatigue), while healthy control subjects did not report any differences in fatigue on these measures. However, there was no significant relation between the GMV changes and changes in these subjective ratings.

Analysis of outliers did not detect any univariate or multivariate outliers. When using a lenient criterion for univariate outlier detection ($|Z| > 2$) there were two outliers in our analysis of changes in GMV ($Z = –2.18$ and $Z = 2.24$), corresponding to one patient showing a GM decrease of −18.5 ml and one patient showing a GM increase of 28.5 ml. When removing these outliers, the relationship between the dynamics in CRT and GMV, as well as between age and the increase in GMV were still significant or still tended to significance, while the correlation between changes in WAIS-dst and GMV was no longer significant. The same was true when using a lenient criterion for multivariate outlier detection [$p(D^2) < 0.05$].

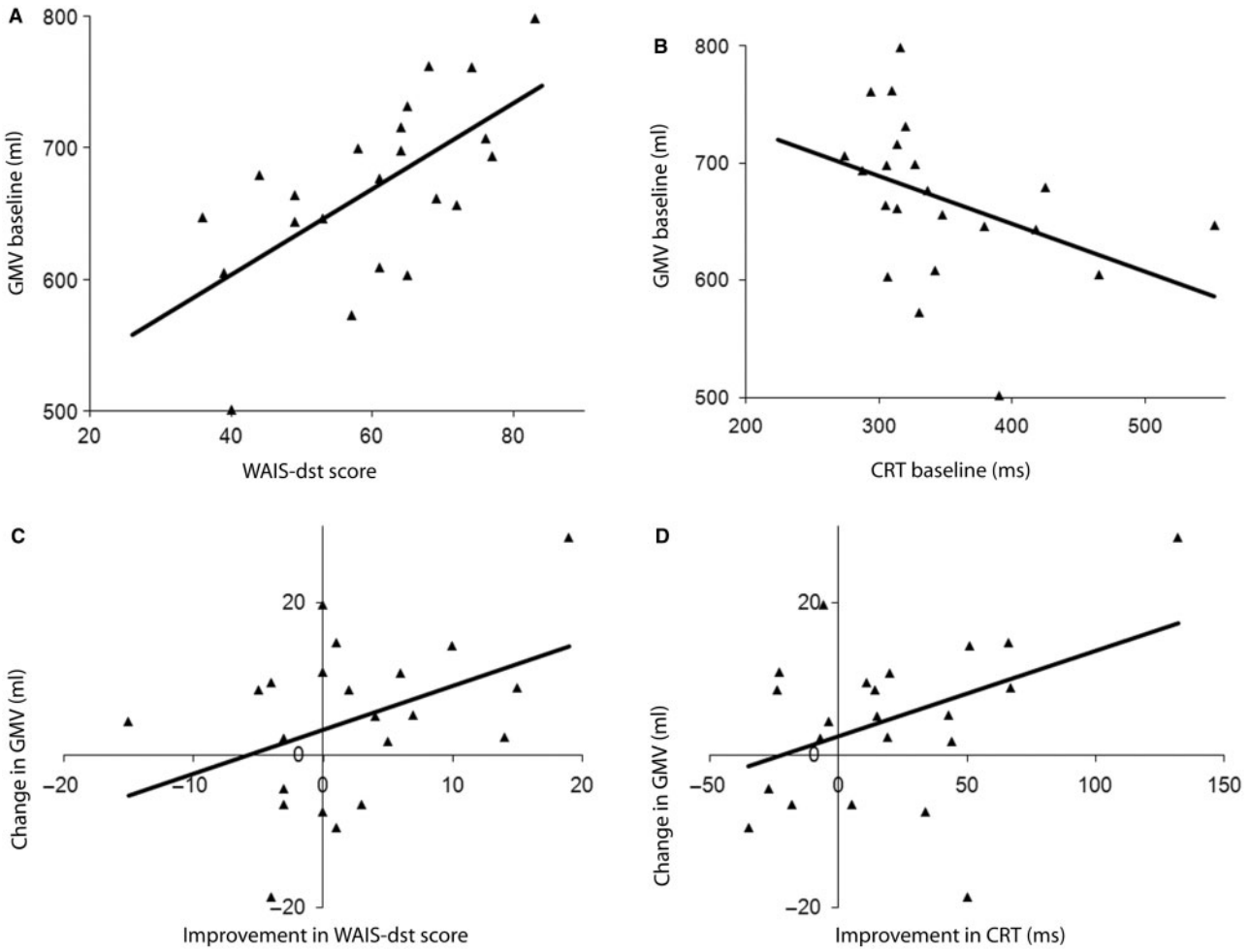

Fig. 4 Correlation between cognitive speed and GMV within the CFS patient group. There is a significant correlation between cognitive speed and GMV at baseline: (**A**) WAIS-dst; and (**B**) CRT. There is also a significant correlation between the *improvement* in cognitive speed and the *increase* in GMV: (**C**) WAIS-dst and (**D**) CRT.

Discussion

In this study we assessed the cerebral plasticity of grey matter atrophy previously observed in CFS patients. The reduction in GMV observed in these patients was partially reversed following the behavioural improvements associated with CBT.

Baseline findings

Confirming previous observations (Okada *et al.*, 2004; de Lange *et al.*, 2005) we found that CFS patients exhibited reduced cerebral GMV compared to healthy control subjects, amounting to a reduction of ~5%. Within the CFS patient group, differences in physical activity had a cerebral counterpart: Physically passive CFS patients had lower GMV than relatively active CFS patients, in line with an earlier study (de Lange *et al.*, 2005). This observation fits with reports of a down-regulation of cell proliferation and neurogenesis in sedentary rodents, as compared to rodents who were in an environment that allowed them to exert physical exercise (van Praag *et al.*, 1999). Functional neuroplasticity related to cardiovascular exercise has also been found in humans (Colcombe *et al.*, 2004).

However, the GMV changes were not exclusively driven by reduction in physical activity. We found that CFS patients with low speed of information processing showed lower GMV. To our knowledge, a relation between information processing speed and GMV has not been documented before. It remains to be seen whether speed of information processing could influence cerebral structure independently from the level of physical activity, and whether a similar relationship between cognitive performance and GMV can be found in healthy controls.

Treatment effects

Following CBT, CFS patients showed significant improvements in health status, physical activity and cognitive performance. These behavioural and cognitive changes had a cerebral counterpart: the CFS patient group revealed a significant GMV increase. Furthermore, the increase in GMV bore relation to the improvement in information processing speed, i.e. bigger improvements in information processing speed were related to larger increases in GMV. The increase in GMV fits with numerous reports on up-regulation of neuroplasticity in rodents and monkeys following enrichment of the environment (Rosenzweig and Bennett 1996; Gross, 2000; van Praag *et al.*, 2000). The macroscopic effects we report arise from a comparison of the average magnetic properties of tens of thousands of neurons and glial cells. Accordingly, these effects can be the result of neuroplastic changes occurring at several levels, including dendritic spine density, dendritic length and branching complexity, expression of spine and synapse-related proteins. Given that neurogenesis in adult mammalian neocortex appears negligible, if present at all (Bhardwaj *et al.*, 2006), it appears reasonable to interpret our findings as an instance of dendritic growth and/or synaptogenesis following environmental enrichment.

The increase in GMV was localized to the lateral prefrontal cortex, within cyto-architectonically defined Brodmann Area 46/9 (Rajkowska and Goldman-Rakic, 1995). In primates, neuroplasticity following environmental enrichment has been observed in the prefrontal cortex (Kozorovitskiy *et al.*, 2005), as an up-regulation of the overall number of spines and presynaptic protein levels, probably reflecting an overall increase in the number of excitatory synapses. It appears plausible that the effects we observe may be a macroscopic manifestation of similar processes, induced by the increase in environmental complexity evoked by CBT.

The lateral prefrontal cortex is an essential node of the network subserving executive functions, as shown by experimental work from neuropsychology, functional imaging in humans, as well as lesion and single-cell recording studies in the monkey (Miller and Cohen, 2001). Lesions in lateral prefrontal cortex often lead to significant reductions in the generation of appropriate goal-directed voluntary behaviour (Jacobsen, 1935; Goldman-Rakic, 1987; Passingham *et al.*, 2005), which can clinically manifest itself as apathy (Levy and Dubois, 2006). The aim of CBT for CFS patients is to establish and maintain a basic amount of physical activity, to plan work or other personal activities, and to gain control over their symptoms (Prins *et al.*, 2006). Given the overlap between the symptoms associated with lateral prefrontal cortical damage and the behaviour that CBT aims to reduce, it appears hardly coincidental that the degree of success of CBT outcome was related to the degree of recovery in grey matter volume in the lateral prefrontal cortex.

Age effects

In line with previous studies (Pfefferbaum *et al.*, 1994; Good *et al.*, 2001), we observed a decrease in GMV with age. Interestingly, we also observed that the post-treatment increase in GMV was dependent on the age of the patient: older patients showed less improvement (or even a decrease) in GMV. This finding suggests that the age-dependent reduction in neuroplastic potential, already documented in animal studies (Kuhn *et al.*, 1996), is also present in humans.

Limitations

Although there was a significant increase in GMV in the CFS patient group, this increase in GMV was a relatively modest portion (12%) of the overall difference in GMV between CFS patients and controls. This finding does not necessarily imply that a substantial portion of the GMV reduction observed in CFS patients is irreversible, even after disease recovery. Namely, not all patients benefited from CBT, and some patients were not yet fully recovered at the time of scanning. Moreover, the GMV increase was dependent on the age of the participant: older CFS patients did not show any increase in GMV, thereby attenuating the

GMV increase observed in the group. Finally, it is conceivable that the cellular and neurophysiological changes follow a slower dynamic than the behavioural improvements. Future studies are needed that follow patients in different age categories at multiple time points over several years to quantify the reversibility of GMV changes following disease recovery. Another limitation of this study is that we did not compare CFS patients treated with CBT with CFS patients that did not undergo CBT treatment. Therefore, we cannot exclude that the cerebral alterations are due to non-specific factors other than the CBT treatment. However, the specific effectivity of CBT, compared to other treatments, has been well-established earlier (Prins *et al.*, 2001; Whiting *et al.*, 2001; Edmonds *et al.*, 2004; Stulemeijer *et al.*, 2005), suggesting that the behavioural effects are likely to be the result of CBT, rather than other unspecific factors.

It may seem somewhat surprising that although we found global differences in GMV between CFS patients and healthy controls, as well as a significant difference between the groups in the increase in global GMV after treatment, we did not find any significant between-groups differences in regional GMV at baseline or follow-up. It is likely that these differences in the results of global and local GMV effects reflect the different statistical sensitivity of these measures. First, global GMV provides a single data-point per subject and therefore it does not require a correction for multiple comparisons. In contrast, local GMV provides a large number of data-points per subject. Statistical inferences on this measure need to be corrected for multiple comparisons, a conservative procedure (Worsley *et al.* 1996; Genovese *et al.*, 2002). Second, global GMV is the integral of local GMV throughout the cortex, i.e. the combined result of regional effects. Assuming a consistent direction of change of the regional effects, global GMV is a more sensitive index than local effects. Third, global GMV is not influenced by the inter-subject variations in brain morphology (that remain even after spatial normalization, see (Ashburner and Friston, 1999, 2000). In contrast, local GMV effects are influenced by the degree of spatial overlap of a given effect across subjects. Taken together, these considerations point to a different sensitivity of local versus global GMV measures, and this is likely to account for the fact that at baseline we could show lower global GMV in the CFS patients, but no supra-threshold regional differences in GMV.

A final limitation of the current study may be that we did not control the time of menstrual cycle in the acquisition of anatomical measurements. This factor posits a small but measurable contribution to MRI images. However, menstrual cycle affects cerebro-spinal fluid volume (Grant *et al.*, 1988; Bydder and Hajnal, 1997), rather than GMV. Moreover, it seems unlikely that there were any systematic differences in menstrual cycle between the groups at baseline or follow-up. Therefore, the lack of control of the menstrual cycle is unlikely to have systematically biased our results.

Conclusions

Our findings indicate that the cerebral atrophy associated with CFS is partially reversed after effective CBT. This demonstrates the surprisingly dynamic relation between behavioural state and cerebral atrophy and points to a potential neurobiological mechanism of cognitive behavioural treatment.

References

Ashburner J, Friston KJ. Nonlinear spatial normalization using basis functions. Hum Brain Mapp 1999; 7: 254–66.

Ashburner J, Friston KJ. Voxel-based morphometry–the methods. Neuroimage 2000; 11: 805–21.

Bergner M, Bobbitt RA, Carter WB, Gilson BS. The sickness impact profile: development and final revision of a health status measure. Med Care 1981; 19: 787–805.

Bhardwaj RD, Curtis MA, Spalding KL, Buchholz BA, Fink D, Bjork-Eriksson T, et al. Neocortical neurogenesis in humans is restricted to development. Proc Natl Acad Sci USA 2006; 103: 12564–8.

Bleijenberg G, Prins J, Bazelmans E. Cognitive-behavioral therapies. In: Jason L, Fennel P, Taylor R, editors. Handbook of chronic fatigue syndrome. New Jersey: John Wiley; 2003a.

Brant-Zawadzki M, Gillan GD, Nitz WR. MP RAGE: a three-dimensional, T1-weighted, gradient-echo sequence–initial experience in the brain. Radiology 1992; 182: 769–75.

Buchwald D, Cheney PR, Peterson DL, Henry B, Wormsley SB, Geiger A, et al. A chronic illness characterized by fatigue, neurologic and immunologic disorders, and active human herpesvirus type 6 infection. Ann Intern Med 1992; 116: 103–13.

Buonomano DV, Merzenich MM. Cortical plasticity: from synapses to maps. Annu Rev Neurosci 1998; 21: 149–86.

Bydder GM, Hajnal JV. Registration and subtraction of serial magnetic resonance images. Part 3: Applications. In: Bradley WG, Bydder GM, editors. Advanced MR imaging techniques. London: Martin Dunitz; 1997. p. 259–79.

Chard DT, Parker GJ, Griffin CM, Thompson AJ, Miller DH. The reproducibility and sensitivity of brain tissue volume measurements derived from an SPM-based segmentation methodology. J Magn Reson Imaging 2002; 15: 259–67.

Colcombe SJ, Kramer AF, Erickson KI, Scalf P, McAuley E, Cohen NJ, et al. Cardiovascular fitness, cortical plasticity, and aging. Proc Natl Acad Sci USA 2004; 101: 3316–21.

Cope H, David AS. Neuroimaging in chronic fatigue syndrome. J Neurol Neurosurg Psychiatry 1996; 60: 471–3.

Cope H, Pernet A, Kendall B, David A. Cognitive functioning and magnetic resonance imaging in chronic fatigue. Br J Psychiatry 1995; 167: 86–94.

de Bruin AF, de Witte LP, Stevens F, Diederiks JP. Sickness impact profile: the state of the art of a generic functional status measure. Soc Sci Med 1992; 35: 1003–14.

de Lange FP, Kalkman JS, Bleijenberg G, Hagoort P, van der Meer JW, Toni I. Gray matter volume reduction in the chronic fatigue syndrome. Neuroimage 2005; 26: 777–81.

Deichmann R, Good CD, Josephs O, Ashburner J, Turner R. Optimization of 3-D MP-RAGE sequences for structural brain imaging. Neuroimage 2000; 12: 112–27.

Dittner AJ, Wessely SC, Brown RG. The assessment of fatigue: a practical guide for clinicians and researchers. J Psychosom Res 2004; 56: 157–70.

Draganski B, Gaser C, Busch V, Schuierer G, Bogdahn U, May A. Neuroplasticity: changes in grey matter induced by training. Nature 2004; 427: 311–2.

Edmonds M, McGuire H, Price J. Exercise therapy for chronic fatigue syndrome. Cochrane Database Syst Rev 2004; CD003200.

Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann Intern Med 1994; 121: 953–9.

Genovese CR, Lazar NA, Nichols T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage 2002; 15: 870–8.

Goldman-Rakic P. Circuitry of primate prefrontal cortex and regulation of behaviour by representational memory. The nervous system. Bethesda: American Physiological Society; 1987. p. 373–417.

Good CD, Johnsrude IS, Ashburner J, Henson RNA, Friston KJ, Frackowiak RSJ. A voxel-based morphometric study of ageing in 465 normal adult human brains. NeuroImage 2001; 14: 21–36.

Grant R, Condon B, Lawrence A, Hadley DM, Patterson J, Bone I, et al. Is cranial CSF volume under hormonal influence? An MR study. J Comput Assist Tomogr 1988; 12: 36–9.

Gross CG. Neurogenesis in the adult brain: death of a dogma. Nat Rev Neurosci 2000; 1: 67–73.

Gur RC, Mozley PD, Resnick SM, Gottlieb GL, Kohn M, Zimmerman R, et al. Gender differences in age effect on brain atrophy measured by magnetic resonance imaging. Proc Natl Acad Sci USA 1991; 88: 2845–9.

Jacobsen CF. Functions of frontal association area in primates. Arch Neurol Psychiatry 1935; 33: 558–69.

Kozorovitskiy Y, Gross CG, Kopil C, Battaglia L, McBreen M, Stranahan AM, et al. Experience induces structural and biochemical changes in the adult primate brain. Proc Natl Acad Sci USA 2005; 102: 17478–82.

Kuhn HG, Dickinson-Anson H, Gage FH. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J Neurosci 1996; 16: 2027–33.

Lange G, DeLuca J, Maldjian JA, Lee H, Tiersky LA, Natelson BH. Brain MRI abnormalities exist in a subset of patients with chronic fatigue syndrome. J Neurol Sci 1999; 171: 3–7.

Levy R, Dubois B. Apathy and the functional anatomy of the prefrontal cortex-basal ganglia circuits. Cereb Cortex 2006; 16: 916–28.

Miller EK, Cohen JD. An integrative theory of prefrontal cortex function. Annu Rev Neurosci 2001; 24: 167–202.

Natelson BH, Cohen JM, Brassloff I, Lee HJ. A controlled study of brain magnetic resonance imaging in patients with the chronic fatigue syndrome. J Neurol Sci 1993; 120: 213–7.

Okada T, Tanaka M, Kuratsune H, Watanabe Y, Sadato N. Mechanisms underlying fatigue: a voxel-based morphometric study of chronic fatigue syndrome. BMC Neurol 2004; 4: 14.

Passingham RE, Rowe JB, Sakai K. Prefrontal cortex and attention to action. In: Humphreys G, Riddoch MJ, editors. Attention in action. Hove: Psychology Press; 2005. p. 263–86.

Pfefferbaum A, Mathalon DH, Sullivan EV, Rawles JM, Zipursky RB, Lim KO. A quantitative magnetic resonance imaging study of changes in brain morphology from infancy to late adulthood. Arch Neurol 1994; 51: 874–87.

Prins JB, Bleijenberg G, Bazelmans E, Elving LD, de Boo TM, Severens JL, et al. Cognitive behaviour therapy for chronic fatigue syndrome: a multicentre randomised controlled trial. Lancet 2001; 357: 841–7.

Prins JB, van der Meer JW, Bleijenberg G. Chronic fatigue syndrome. Lancet 2006; 367: 346–55.

Rajkowska G, Goldman-Rakic PS. Cytoarchitectonic definition of prefrontal areas in the normal human cortex: II. Variability in locations of areas 9 and 46 and relationship to the Talairach Coordinate System. Cereb Cortex 1995; 5: 323–37.

Rosenzweig MR, Bennett EL. Psychobiology of plasticity: effects of training and experience on brain and behavior. Behav Brain Res 1996; 78: 57–65.

Schwartz RB, Garada BM, Komaroff AL, Tice HM, Gleit M, Jolesz FA, et al. Detection of intracranial abnormalities in patients with chronic fatigue syndrome: comparison of MR imaging and SPECT. AJR Am J Roentgenol 1994; 162: 935–41.

Stulemeijer M, de Jong LW, Fiselier TJ, Hoogveld SW, Bleijenberg G. Cognitive behaviour therapy for adolescents with chronic fatigue syndrome: randomised controlled trial. BMJ 2005; 330: 14.

Talairach J, Tournoux P. Co-planar stereotaxic atlas of the human brain: 3-dimensional proportional system: an approach to medical cerebral imaging. Stuttgart: Thieme; 1988.

van der Werf SP, Prins JB, Vercoulen JH, van der Meer JW, Bleijenberg G. Identifying physical activity patterns in chronic fatigue syndrome using actigraphic assessment. J Psychosom Res 2000; 49: 373–9.

van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 1999; 2: 266–70.

van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. Nat Rev Neurosci 2000; 1: 191–8.

Vercoulen JH, Bazelmans E, Swanink CM, Fennis JF, Galama JM, Jongen PJ, et al. Physical activity in chronic fatigue syndrome: assessment and its role in fatigue. J Psychiatr Res 1997; 31: 661–73.

Vercoulen JH, Bazelmans E, Swanink CM, Galama JM, Fennis JF, van der Meer JW, et al. Evaluating neuropsychological impairment in chronic fatigue syndrome. J Clin Exp Neuropsychol 1998; 20: 144–56.

Vercoulen JH, Swanink CM, Fennis JF, Galama JM, van der Meer JW, Bleijenberg G. Dimensional assessment of chronic fatigue syndrome. J Psychosom Res 1994; 38: 383–92.

Wechsler D. WAIS-R, Wechsler Adult Intelligence Scale Revised. New York: The Psychological Corporation; 1981.

Wen W, Sachdev PS, Chen X, Anstey K. Gray matter reduction is correlated with white matter hyperintensity volume: a voxel-based morphometric study in a large epidemiological sample. Neuroimage 2006; 29: 1031–9.

Whiting P, Bagnall AM, Sowden AJ, Cornell JE, Mulrow CD, Ramirez G. Interventions for the treatment and management of chronic fatigue syndrome: a systematic review. JAMA 2001; 286: 1360–8.

Worsley KJ, Marrett S, Neelin P, Vandal AC, Friston KJ, Evans AC. A unified statistical approach or determining significant signals in images of cerebral activation. Hum Brain Mapp 1996; 4: 58–73.