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Reduced oxygen due to high-altitude exposure relates to atrophy in motor-function brain areas

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At high altitudes barometric pressure is reduced and, thus, less oxygen is inhaled. Reduced oxygen concentration in brain tissue can lead to cerebral damage and neurological and cognitive deficits. The present study was designed to explore the effects of high-altitude exposure using a quantitative MRI technique, voxel-based morphometry.We studied nine world-class mountain climbers before (baseline) and after (follow-up) an extremely high-altitude ascent of Everest and K2. We investigated the effects of repeated extremely high-altitude exposures by comparing mountain climbers' scans at baseline with scans of 19 controls. In addition, we measured the effects of a single extremely high-altitude expedition by comparing mountain climbers' scans at baseline and follow-up.A region of reduced white matter density/volume was found in the left pyramidal tract near the primary (BA 4) and supplementary (BA 6) motor cortex when mountain climbers at baseline were compared with controls. Further, when mountain climbers' scans before and after the expedition were compared, a region of reduced grey matter density/volume was found in the left angular gyrus (BA 39). These findings suggest that extremely high-altitude exposures may cause subtle white and grey matter changes that mainly affect brain regions involved in motor activity.

Introduction

Several studies have reported that high-altitude exposure (above 5000 m) is a potential cause of transient or permanent neurological deficits, cognitive dysfunctions, and brain abnormalities detected on MRI [1,2]. These high-altitude effects are mainly ascribed to oxygen deprivation (hypoxia) in tissues, including the blood. High-altitude exposure is a particular case in which hypoxia is not due to a reduced quantity of oxygen in the environment but to a decrease in barometric pressure resulting in less oxygen inhalation.

Results of previous MRI studies of brain changes following high-altitude climbs are inconsistent. Garrido *et al*. [3,4] and Fayed *et al*. [5] detected diffuse cortical atrophy and white matter hyperintensity in climbers who scaled over 7000 m. 'High-altitude cerebral edema' (white matter edema) was reported to be reversible by Hackett *et al*. [6], Jeong *et al*. [7] and Usui *et al*. [8] reported three cases of 'high-altitude cerebral edema' that showed bilateral lesions in the globus pallidus. However, Anooshiravani *et al*. [9] did not observe any brain changes in their group, which scaled 7100 m.

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There may be various reasons for these inconsistencies: different high-altitude experiences of climbers, manifestation of neurological disturbances during or after the expedition, the use of different MRI techniques to analyze the imaging data. In fact, the studies mentioned used conventional MRI to investigate brain changes after high-altitude exposure; therefore, they provide only qualitative or semi-quantitative indexes of brain damage. Hence, with the aim of collecting quantitative information on regional brain abnormalities we applied a quantitative MRI technique, voxel-based morphometry (VBM). VBM is a spatially-specific and unbiased method that is completely operator independent. This technique allows studying the whole brain with no *a priori* hypothesis about which brain regions might be affected by high-altitude hypoxia. It provides quantitative information on regional volume or density reductions of grey matter (GM) and white matter (WM) in voxel scale [10].

To our knowledge, this is the first study that used VBM to investigate a group of world-class mountain climbers before and after a high-altitude expedition to the top of Mount Everest (8848 m) and K2 (8611 m) without an oxygen supply. This expedition, which had both scientific and athletic goals, was undertaken to mark the 50th anniversary of the first K2 ascent.

The aims of this study were the following:

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(i) to evaluate and quantify regional GM and WM changes after repeated expeditions;
(ii) to evaluate GM and WM changes detectable after a single expedition; and
(iii) to investigate whether measures of regional GM density correlate with neuropsychological assessments.

Materials and Methods

Subjects

A group of nine world-class mountain climbers [all men; mean age in years (SD) = 37.9 (7.2); range = 31–52 years; all right handed] were recruited. All subjects had been mountain climbing for at least 10 years before participating in this study and were accustomed to reaching altitudes over 4000 m several times a year in the Alps. The control group was comprised of 19 sex- and age-matched healthy subjects. They had no history of high-altitude exposure (i.e. over 3000 m) and they had normal daily physical activity without special physical training. All subjects (climbers and controls) were carefully checked to exclude the presence of any major systemic, psychiatric, or neurological illnesses. Then they were subjected to a research protocol that included MRI and neuropsychological testing. Mountain climbers were first studied eight weeks before the expedition began (baseline) [mean (SD) time in weeks (SD) = 7 (1)]. One climber reached the top of both Everest and K2; two reached the top of either Everest or K2; all the other climbers reached altitudes of over 7500 m and spent at least fifteen days at altitudes over 6500 m. The mountain climbers who did not reach the highest altitude had been affected by Acute Mountain Sickness (AMS). According to the Lake Louise Consensus Committee Definition of AMS [11], the medical team present during the ascent verified the presence of the three basic criteria: a recent gain in altitude, at least several hours at the new altitude, presence of headache, and the occurrence of at least one of the following symptoms: gastrointestinal upset, fatigue or weakness, dizziness or light-headedness, difficulty sleeping, i.e. all of the manifestations necessary to make the diagnosis of AMS. Six out of nine climbers diagnosed with AMS were advised to stop the ascent and to return to the base camp, which was located at a lower altitude.

All climbers were reassessed 8 weeks after they returned from the expedition [mean time in weeks (SD) = 7.6 (0.7)]. They were subjected to the same MRI and neuropsychological protocol used during the first evaluation. The control group underwent a single study session at baseline.

Local Ethical Committee approval and written informed consent were obtained before the study began.

Neuropsychological assessments

The previous studies that investigated changes in cognitive profile secondary to high-altitude exposure generally reported the effects of altitude exposure on motor, memory, and 'frontal-lobe' function in adults, including language and executive functions [2,12]. Accordingly, we chose the following neuropsychological battery to assess the cognitive profile of our group of mountain climbers:

(i) the Prose Memory Test (immediate and delayed recall) and the Rey-Osterrieth Complex Figure Test (copy, immediate, and delayed recall) for verbal and visuo-spatial episodic memory [13,14];
(ii) the Block Design [15] and the Benton Judgment of Line Orientation Test [16] for visuo-motor and visuo-spatial functions; and
(iii) the forward Digit Span [15], the Digit Symbol [15] and the Phonological Word Fluency test (FAS fluency) [17] for working memory, executive functions and language.

All neuropsychological test scores were corrected for *age*, education, and gender [18–20].

We expected to find the same cognitive alterations in our mountain climbers as those found in previous studies (i.e., primarily low memory and language performance on neuropsychological tests).

MRI

MRI acquisition

All MRI data were acquired using an MR scanner operating at 1.5 T (Siemens, Magnetom Vision, Erlangen, Germany). The following pulse sequences were obtained in a single session:

(i) axial T2-weighted fast spin-echo (SE) (TR/TE: 3800/90 ms);
(ii) axial fluid-attenuated inversion-recovery (FLAIR) (TR/TE: 9000/119 ms; TI: 2470 ms); and
(iii) 3D T1-weighted magnetization-prepared rapid-acquisition gradient echo (MPRAGE) (TR/TE: 11.4/4.4 ms; TI: 20 ms; flip angle: 15°).

For the T2-weighted and FLAIR sequences, 21 axial slices, 5 mm-thick, with an intersection gap of 1 mm, a 240 mm field of view, and a 256 × 256 matrix were acquired. For the MPRAGE sequence, 159 slices, 1 mm-thick, with sagittal orientation, a 256 × 256 matrix size, and a 256 mm field of view were acquired.

MRI analysis and post-processing

Two experienced observers (M.B. and U.S.), unaware of whom the scans belonged to, independently reviewed the T2-weighted and FLAIR scans of all

subjects to identify, by consensus, pathological hyperintensities.

The MPRAGE images were processed using SPM2 (Wellcome Dept. Cogn. Neurol., London; http://www.fil.ion.ucl.ac.uk/spm). Briefly, to optimize brain extraction and tissue segmentation these images underwent an iterative procedure. According to the optimized VBM approach [10], the GM and WM extracted in native space were normalized respectively to the GM and WM template of SPM (using a combination of linear and non-linear functions), and the optimized transformation parameters were then applied to the original T1-volumes in native space. Finally, the normalized images were segmented again, producing GM, WM, and cerebrospinal fluid (CSF) maps. The signal intensity in every voxel of these maps represents the probability of belonging to a given class of tissue and reflects the regional density of such tissue. The GM and WM images were also modulated and they were smoothed with10 mm^3 FWHM Gaussian kernel. Statistical analysis was performed both on regional GM and WM density (un-modulated images) and volume (modulated images) maps.

Normalized whole-brain and segmented images obtained from the automated VBM processing for each mountain climber and control were then compared visually with the template image to verify the efficacy of normalization. Moreover, for the climbers' within group analysis, we also checked the images one by one before and after the expedition to exclude the presence of misleading alignment among the brains.

Before testing for regional changes, we investigated (using all scans obtained from mountain climbers and controls) whether the average whole brain volumes were significantly different in the two groups. The whole brain volume was calculated for each subject using the number of voxels belonging to WM and GM probability maps. Student's t-test for unpaired data was used to compare the two groups.

The changes due to repeated extremely high-altitude exposures were first tested using an ANOVA model, for GM and WM respectively, which included all scans obtained at baseline. Comparisons between the two groups (mountain climbers and controls) were made to investigate regional GM and WM differences. For each ANOVA model, age and intracranial GM or WM volume were entered as covariates to correct for potential confounds.

Analysis of GM matter was performed on the normalized and smoothed GM density and volume map of the whole brain. Thus, all GM voxels of each brain (mountain climbers and control subjects) were included in the analysis, without choosing any *a priori* region of interest.

We followed the same procedure when we analyzed the WM matter. We chose the normalized and smoothed WM density and volume map of the whole brain for each subject (mountain climbers and control subjects) without choosing any *a priori* region of interest.

Moreover, GM and WM volume (included in the matrix as covariates) was calculated for each subject using the number of voxels belonging to the GM or the WM normalized and smoothed map obtained from VBM analysis.

The changes due to a single extremely high-altitude exposure were evaluated using a paired *t*-test model, for GM and WM respectively, to compare images obtained from climbers before (baseline scans) and after the expedition (follow-up scans). For any contrast, the statistical threshold was set to $P < 0.05$, corrected at the cluster level, as in previous VBM studies [21–24]. Because of the inconsistent results in the literature on the cerebral effect of high altitude on mountain climbers, we conferred an exploratory nature to this investigation and we thresholded the SPM maps at the $P < 0.05$ cluster-level, corrected for multiple comparisons. Then, to increase confidence in obtaining genuine results and to reduce the risk of false-positives we corrected the cluster threshold with the spatial extent [25–27]. Thus, for the WM analysis only those regions with a spatial extent equal to or greater than 1137 voxels were considered significant, and for the GM analysis only those with a size equal to or greater than 1077 voxels.

Finally, an ANOVA was performed to investigate correlations between regional GM density and neuropsychological measures obtained from mountain climbers.

In all tables, stereotaxic coordinates were reported in Montreal Neurological Institute (MNI) space [28]. These coordinates match the Talairach and Tournoux [29] atlas closely, but not exactly.

Results

Neuropsychological assessments

Neuropsychological data obtained from mountain climbers at baseline are summarized in Table 1. Some subjects obtained scores below the normal cut-off on the neuropsychological tests. Such abnormalities remained substantially unchanged at follow-up, thus suggesting there were no significant changes consequent to the expedition.

MRI

No recruited subject from either group (mountain climbers or controls) showed abnormalities on T2-

Table 1 Neuropsychological assessments obtained at baseline from mountain climbers

	Mean (SD) [range]	Cut-off?	Number (%) of subjects With neuropsychological scores under cut-off
Prose memory immediate recall score	4.2 (2.4) [0.0–6.4]	≤3.1	3 of 9 (33.3%)
Prose memory delayed recall score	4.1 (1.2) [2.8–5.9]	≤2.4	0 of 9 (0.0%)
Rey's figure immediate copy score	29.7 (5.1) [19.3–36.0]	≤23.7	1 of 9 (11.0%)
Rey's figure immediate recall score	11.8 (9.1) [−1.3–29.0]	≤6.4	2 of 9 (22.2%)
Rey's figure delayed recall score	10.1 (8.1) [1.4–29.0]	≤6.3	3 of 9 (33.3%)
Digit span score	5.3 (0.5) [4.5–6.0]	≤3.7	0 of 9 (0.0%)
Phonological word fluency (FAS) score	28.9 (8.4) [16.8–41.5]	≤17.3	1 of 9 (11.0%)
Digit symbol score	8.4 (3.3) [4.0–13.0]	≤10.0	6 of 9 (66.7%)
Block design score	11.2 (2.9) [6.0–13.0]	≤10.0	4 of 9 (44.4%)
Judgment of line orientation score	26.3 (3.1) [21.0–30.0]	≤25.6	2 of 9 (22.2%)

We report the mean (SD) [range] scores from each test administered to mountain climbers at baseline; all neuropsychological scores are corrected for age and level of education. For each test, the cutting score, defined as the lower limit of the 90% tolerance interval around the normative mean is reported. The number (%) of subjects who obtained scores under the cut-off is also reported.

weighted and FLAIR scans. No MPRAGE scans were affected by artifacts, as assessed by visual examination.

In particular, we were interested in checking whether focal hyperintensity signals were present in the brain tissue. This was carried out by an expert neuro-radiologist who visually inspected the T2-weighted and FLAIR sequences. The neuroimaging evaluation (carried out after the climbers returned from the expedition) and the clinical report (carried out by the medical team during the climb), consistently indicated the absence of high-altitude cerebral edema and high-altitude pulmonary edema in our group.

There was no significant difference in average whole brain volume between mountain climbers [mean (SD) = 1540 (290) ml] and controls [mean (SD) = 1529 (220) ml] [$P = 0.33$, n.s.].

We obtained the same result with modulated and un-modulated data. VBM analysis to assess changes due to repeated exposure did not show any significant difference in GM density/volume between mountain climbers and controls. By contrast, reduced WM density/volume was found in mountain climbers compared with controls. It involved the left pyramidal tract in a region near the left primary (BA 4) and supplementary (BA 6) motor cortex ($P = 0.003$, corrected at cluster level) (see Table 2A and Fig. 1). This part of the pyramidal tract fits well with the anatomical location of motor fibers subserving the right-hand motor function.

When we tested mountain climbers for changes following a single expedition (follow-up vs. baseline), we found a region of reduced GM density/volume in the left angular gyrus (BA 39) ($P = 0.002$, corrected at cluster level) (see Table 2B and Fig. 2). However, we found no significant change in WM density/volume.

We further investigated whether, in mountain climbers, there was any correlation between regional GM density/volume and scores obtained on the neuropsychological tests. For this purpose, we created an additional ANOVA model that included GM maps and scores obtained on neuropsychological tests at baseline. No statistically significant correlation was found between regional GM density/volume and scores obtained on any neuropsychological tests.

Discussion

This study was designed to investigate the repeated and the single effects of extremely high-altitude exposures in a world-class group of mountain climbers using VBM. All recruited subjects had been professional climbers for at least 10 years prior to the study.

Table 2 Regions of significantly reduced WM and GM density in mountain climbers

				Coordinates MNI				
Brain region	GM/WM	Side	Size	x	y	z	Peak Z-score	*P* value*
(A) Repeated exposure effect								
Pyramidal tract	WM	L	4131	−33	−15	54	3.93	0.003
(B) Single exposure effect								
Angular Gyrus	GM	L	1130	−53	−65	23	4.28	0.002

*$P < 0.05$, corrected at cluster level; we reported coordinates and voxel size of un-modulated data; The size of each region is expressed in number of voxels; Table 2 (A) shows the effects of repeated exposure; Table 2 (B) shows the effects observed after a single exposure.

LOW RESOLUTION COLOUR FIG

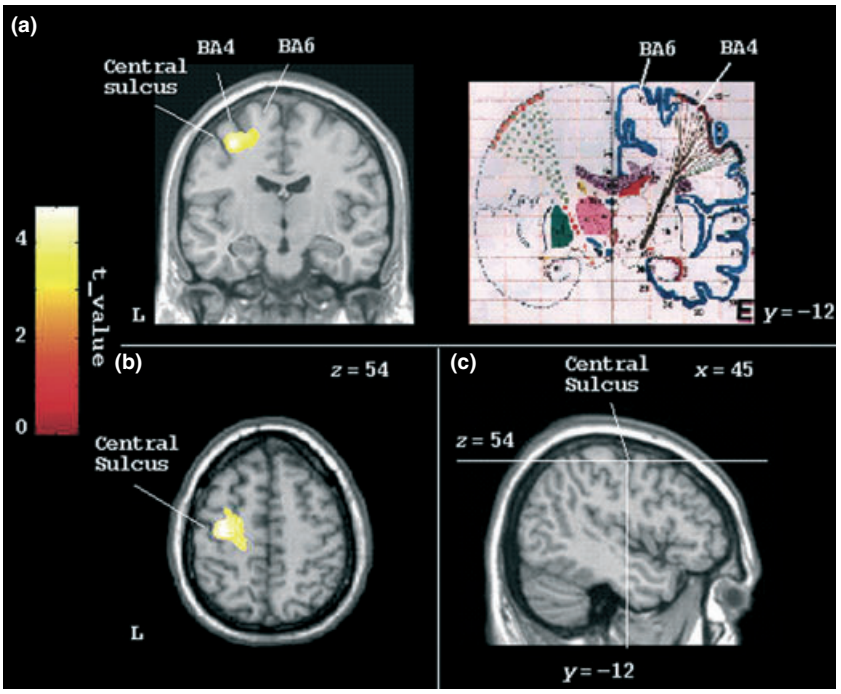

Figure 1 Regional reduction in white matter density at baseline of mountain climbers compared with controls. The area involves the left pyramidal tract near primary (BA 4) and supplementary (BA 6) motor cortex. Panel (a) shows the thresholded map of *t*-statistic values (coronal section, y = −12) of this region superimposed on the single subject T1-weighted normalized brain of SPM (left side). The corresponding slice from the Talairach and Tournoux atlas (right side) shows the pyramidal tract. Panel (b) shows the thresholded map of *t*-statistic values of the same region (axial section, z = 54). Panel (c) shows the position of the slices considered in panels A (coronal) and B (axial) superimposed on the single subject T1-weighted normalized brain of SPM (sagittal section, x = 45). L = left.

LOW RESOLUTION COLOUR FIG

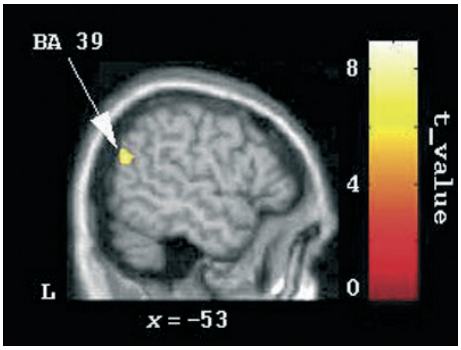

Figure 2 Results of VBM analysis comparing the scans obtained from mountain climbers before (baseline) and after (follow-up) an extremely high-altitude expedition. A reduction in GM density is detectable in the left angular gyrus (BA 39). Here we report the thresholded map of *t*-statistic values (sagittal section, x = −53) of this region superimposed on the single-subject T1-weighted normalized brain of SPM. L = left.

Conventional MRI showed no abnormalities on T2-weighted and FLAIR scans in any of the subjects either before or after the expedition that was monitored in this study. Moreover, no significant differences were found in the average whole brain volume of mountain climbers compared with controls, indicating that no visible global or focal brain changes occurred during any of their repeated extremely high-altitude exposures.

In this study, VBM analysis was used to assess regional differences in GM and WM density and/or volume between mountain climbers and controls. This analysis, which was applied to a group of putatively normal individuals, included the entire brain with no *a priori* assumptions about any regional brain involvement.

First, we investigated the potential effects of repeated extremely high-altitude exposures by comparing scans obtained from world-class climbers before their expedition to Everest and K2 and from controls. A well localized region of significantly reduced WM density/volume was identified that involved the left pyramidal tract near the primary (BA 4) and supplementary (BA 6) motor cortex. This part of the pyramidal tract fits well with the anatomical location of motor fibers subserving the right-hand motor function. As all subjects included in this study were right-handed, this localization might reflect the involvement of fibers that have a role in the right-hand motor function. It is, in fact, reasonable to hypothesize that the right upper limb is used most in climbing.

When we investigated the effect of a single extremely high-altitude exposure, we found a significant region of reduced GM density/volume in the left angular gyrus (BA 39), a highly associative cortical area involved in a number of high-level cognitive functions including cognitive planning of movement [30]. When damage occurs in this area, movements may be characterized by errors in posture, spatial orientation, and joint coordination [31,32]. Once again, it appears that the reduced anatomical density in our mountain climbers regarded a brain region involved in motor skills, in this case high-level motor skills.

It is well known that some brain structures (such as the so-called watershed zones, hippocampus, striatum, thalamus, and amygdale) are more sensitive to oxygen deprivation (such as that which occurs in high altitude environments) than others.

The 'selective vulnerability' of some anatomical regions is due to different factors:

(i) their position in the territory supplied by the major cerebral arteries;
(ii) the specific metabolic and biochemical properties of the structures [33];
(iii) the vulnerability of cell groups; and
(iv) the different response of brain regions over time, with some anatomical regions affected shortly after the event and others a few days later [34].

Hypoxia secondary to both high-altitude cerebral edema and high-altitude pulmonary edema, regardless of the difference in the underlying biological process, should cause focal damage in the GM (i.e. in those regions that are selectively vulnerable) and diffuse damage in the WM (i.e. a diffuse periventricular deep white matte signal change, called leuko-araiosis) [35].

Indeed, there is only limited evidence of focal brain damage in hypoxia-sensitive brain areas (i.e. cortex, medial temporal lobe) and diffuse WM damage resulting from acute exposure to extremely high altitudes. Thus, some experts [12] have suggested that the symptoms described in mountain climbers may be mostly transient and associated only with acute perturbations of brain metabolism. Here, we suggest another explanation for our data. We hypothesize that our results can be explained by a combination of at least two factors:

(i) the brain areas we found vulnerable to hypoxia were located in a 'border zone';
(ii) areas of increased metabolism during climbing require more oxygen in a condition in which oxygen inhalation is reduced.

In a hypoxic condition, like the one that occurs at high altitudes, perfusion (blood flow through an organ) may precipitate, resulting in a severe decrease in blood flow through an organ. As the reduction in blood flow is global, all parts of the brain are affected, especially 'watershed' areas, i.e. border zone regions supplied by the major cerebral arteries. Blood flow to these areas does not necessarily stop; instead, it may lessen to the point where brain damage can occur. This phenomenon is also referred to as 'last meadow' to point to the fact that in irrigation the last meadow receives the least amount of water. Here, the area we found atrophic was a border zone (where the pyramidal tract lies) supplied by the anterior cerebral and the middle cerebral arteries. This observation is in agreement with the result of Rostrup's study [36] (they found that in acute hypoxia condition, there is a significant reduction of the magnitude of the BOLD response in brain visual and motor areas).

We also agree with Hornbein [37] that the imbalance between oxygen supply and need may affect some brain regions. In other words, not only a decrease in oxygen supply (i.e. the normal condition at high altitudes) but also an increase in the need for oxygen (i.e. the normal request during climbing) can explain part of the brain damage. Such focal damage can also occur in the absence of signs of more global brain changes.

Thus, we hypothesize that the imbalance between oxygen required and oxygen inhaled may have caused some subtle neuronal sufferance, particularly in neurons and fibers involved in motor functioning activity that are vulnerable to the hypoxic condition because of their anatomical location.

Sufferance in the cerebral motor area has been found in previous studies. A review of the literature on the reported cases of hypoxic brain injury reveals that motor disorders are the predominant clinical residues after pure hypoxic injury and that they may manifest after significant latency [38–41].

Finally, our data are consistent with previous reports of the persistence of motor impairment in mountain climbers after they return to sea level [42–44]. Indeed, in some cases signs of motor impairment could still be detected even one [45] or two years [46] after a high-altitude expedition. Therefore, we suggest that the pattern of atrophy involving the pyramidal tract and the angular gyrus might explain, at a different level of complexity, the motor deficit affecting mountain climbers.

Although there have been several reports of acute abnormalities in the neuropsychological functioning of mountain climbers [2,4,42,47–53], in our subjects we found no significant worsening of neuropsychological performance after the expedition. However, some of the subjects obtained abnormal scores on the neuropsychological tests (Table 1), but with no significant difference between baseline and follow-up. All were relatively young, and there was no evidence of a pathological condition that could account for such impairment. Moreover, before the study began all had

been carefully checked to exclude any concomitant clinical condition. Thus, the abnormal scores on the neuropsychological tests can be explained as the result of progressive, subtle, brain insults likely due to repeated high-altitude exposures.

The main limitation of the present study is the small number of subjects recruited. Therefore, these results need to be replicated and extended in studies involving larger populations. However, it must be considered that extremely high-altitude mountain climbing is an unusual practice and therefore the recruitment of subjects is particularly difficult. Moreover, these results might be considered as preliminary data and the study as an exploratory attempt to quantify brain changes in mountain climbers.

Another limitation concerns the time window used to reassess mountain climbers after the expedition, which was probably too wide and may account for the poor sensitivity in detecting correlations between imaging and neuropsychological data.

Conclusions

The present study investigated the effects of both repeated and single extremely high-altitude exposures on brain tissue in a group of world-class mountain climbers, using VBM. Our results provide evidence that extremely high-altitude climbs with no external oxygen supply may cause subtle changes in brain tissue, even when well acclimatized individuals do not experience any neurological symptoms. Moreover, these changes seem to be highly specific for some brain regions. These findings suggest that extremely high-altitude exposures may result in subtle white and grey matter changes that mainly affect the brain regions involved in motor activity. This interpretation is consistent with previous reports of long-term motor impairment in mountain climbers after their return to sea level.

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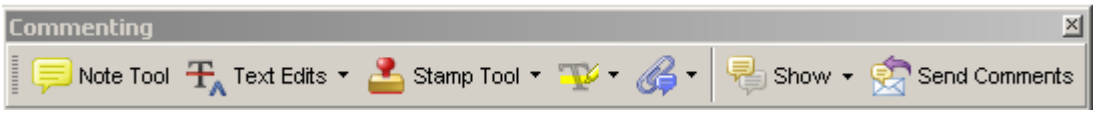
Adobe Acrobat Professional or Acrobat Reader (version 7.0 or above) is required to e-annotate PDFs. Acrobat 8 Reader is a free download: http://www.adobe.com/products/acrobat/readstep2.html

Once you have Acrobat Reader 8 on your PC and open the proof, you will see the Commenting Toolbar (if it does not appear automatically go to Tools>Commenting>Commenting Toolbar). The Commenting Toolbar looks like this:



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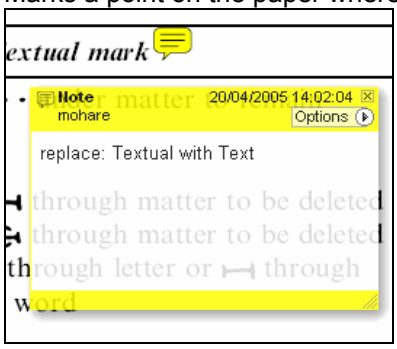
Marks a point on the paper where a note or question needs to be addressed.



How to use it:

1. Right click into area of either inserted text or relevance to note
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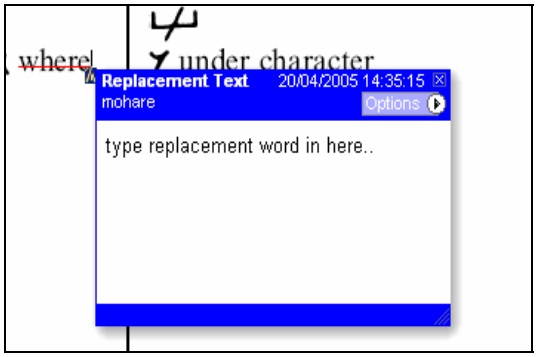
Strikes red line through text and opens up a replacement text box.



How to use it:

1. Select cursor from toolbar
2. Highlight word or sentence
3. Right click
4. Select Replace Text (Comment) option
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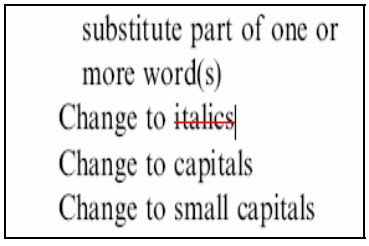
Cross out text tool — For deleting text when there is nothing to replace selection

Strikes through text in a red line.



How to use it:

1. Select cursor from toolbar
2. Highlight word or sentence
3. Right click
4. Select Cross Out Text

Approved tool — For approving a proof and that no corrections at all are required.

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1. Click on the Stamp Tool in the toolbar
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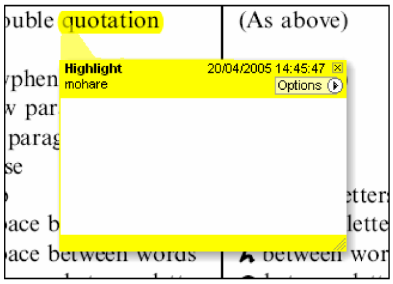
Highlight tool — For highlighting selection that should be changed to bold or italic.

Highlights text in yellow and opens up a text box.



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1. Select Highlighter Tool from the commenting toolbar
2. Highlight the desired text
3. Add a note detailing the required change

Attach File Tool — For inserting large amounts of text or replacement figures as a files.

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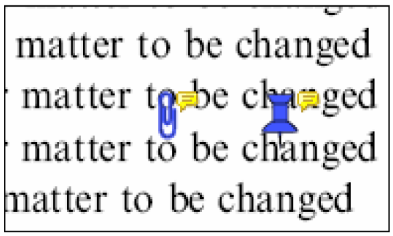

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1. Click on paperclip icon in the commenting toolbar
2. Click where you want to insert the attachment
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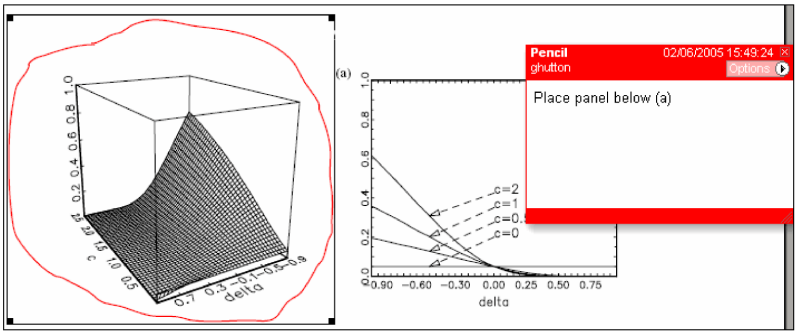

How to use it:

1. Select Tools > Drawing Markups > Pencil Tool
2. Draw with the cursor
3. Multiple pieces of pencil annotation can be grouped together
4. Once finished, move the cursor over the shape until an arrowhead appears and right click
5. Select Open Pop-Up Note and type in a details of required change
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