PET Imaging of Mild Traumatic Brain Injury and Whiplash Associated Disorder
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Altered Regional Cerebral Blood Flow in Chronic Whiplash Associated Disorder

David Vállez García, Andreas Otte, Antoon TM Willemsen, Rudi AJO Dierckx, Janine Doorduin, Gert Holstege

Manuscript in preparation
Abstract

Whiplash Associated Disorder (WAD) is one of the most frequent consequences of motor vehicle accidents. While initial symptoms resolve within a few weeks in many cases, some patients develop persistent symptoms that include pain, headache, visual, and/or psychological disturbances. Although there is evidence supporting tissue lesion and central hyperexcitability, the pathophysiology is not well understood. In the current study, possible alterations in the regional cerebral blood flow (rCBF) were explored with PET imaging. Twelve female patients diagnosed with WAD grade I/II were included 5±2 years after the accident, in addition to eight healthy matched volunteers. PET scans (Ecat HR+, Siemens) were acquired after injection of 500 MBq $[^15]$O$\text{H}_2$O for rCBF measurement. During acquisitions the volunteers received a non-painful electrical stimulation of the neck. Sensitivity thresholds were individually determined. Four conditions were used: rest state, placebo-like state (current was expected but not generated), stimulation above perception threshold, and stimulation below pain or muscular contraction. These conditions were repeated three times, resulting in twelve scans. All participants completed a neurological interview, the Hospital Anxiety and Depression Scale, the Neck Disability Index (NDI), the Whiplash Disability Questionnaire, and rated pain on the visual analogue scale. Voxel-based analysis was performed on the scans with SPM8 in combination with SwE toolbox to account for repeated measurements. Level of significance was set to $p<0.005$ uncorrected, with an extent threshold of 100 voxels. Correlations between the rCBF of WAD patients in the significant clusters and the scores from questionnaires were analyzed using the Generalized Estimating Equations model in SPSS, with $p<0.05$ considered significant. In all the questionnaires WAD patients scored higher ($p<0.001$) than healthy volunteers. No significant differences in rCBF were found between conditions. In WAD patients, compared with healthy controls, a significant increase in the rCBF was found in the right superior parietal gyrus ($Z=2.96\pm0.30$). In addition, a decreased rCBF was found in the left insula ($Z=3.03\pm0.39$), right insula ($Z=3.06\pm0.40$), and right thalamus ($Z=2.94\pm0.27$). Within the WAD group, a negative association ($p<0.009$) was found between the NDI scores and the rCBF. WAD symptoms might be the result of a mismatch between the proprioceptive information from the cervical spinal cord and the information integrated in regions such as mesencephalon, thalamus and insula. However, further investigation of the functional brain alterations present in WAD patients must be performed for a better understanding of the pathophysiology.
Introduction

Whiplash trauma is one of the most frequent consequences of motor vehicle related accidents. It is estimated to have a yearly cost in Europe of at least €10 billion\(^1\) and $29 billion in the United States,\(^2\) affecting about 300 per 100,000 persons per year in North America and Western European countries.\(^3\) While in many cases the initial symptoms resolve within few weeks after the accident, approximately half of whiplash injured individuals will develop persistent symptoms.\(^4\) This heterogeneous group of symptoms is generally defined as Whiplash-associated disorder (WAD),\(^5\) and include pain in the neck and headache as the most frequent symptoms, followed by interscapular and temporomandibular pain, paresthesia in arms and hands, dizziness, visual and psychological disturbances.\(^6\)

While there is evidence supporting lesion of various tissues in WAD, mainly concerning the zygapophysial (facet) joints, most of these injuries are undetected by conventional imaging techniques.\(^7\) Radiography, computed tomography (CT), and magnetic resonance imaging (MRI) are inconclusive for the prognosis of WAD.\(^8\) Aside from the use of standard imaging techniques for clinical diagnosis, the use of functional neuroimaging techniques such positron emission tomography (PET) and single-photon emission computed tomography (SPECT) can help to understand the underlying mechanism of WAD. Otte et al.\(^9,10\) performed a series of PET and SPECT studies in over 500 whiplash patients at rest using different tracers \([99mTc]\)-hexamethyl propylene amine oxime (HMPAO), \([99mTc]\)-ethylene biyldicysteinate dimer (ECD), and \([18F]\)-fluorodeoxyglucose (FDG). Hypoperfusion and hypometabolism in the posterior parietal occipital cortex was observed in many of the patients as compared with healthy control. Similar findings were obtained recently in a PET study that measured the regional cerebral blood flow (rCBF) by means of \([15O]\)H\(_2\)O. Moreover, elevated rCBF was found bilaterally in the posterior parahippocampal, posterior cingulate gyri, right medial prefrontal gyrus, and right thalamus.\(^11\) Other imaging studies support also these results, showing statistical significant reduced perfusion of the temporal lobe in WAD patients,\(^12\) and non-significant (uncorrected voxel level of \(p=0.001\)) small regions with lower rCBF at the left temporoparietal, and right temporal region.\(^13\) However, other studies did not obtain similar results,\(^14,15\) maybe as consequence of the presence of important biases in the study design, e.g. control group composed mainly of melanoma patients.

The current study was design to address three main objectives. First, it intends to replicate the results of previous experiment that shown alterations in the rCBF in the posterior parietal, temporal and occipital lobe, hypothesized to be related with nociceptive afferents causing increased levels of vasopeptide
induced vasoconstriction.\textsuperscript{16,17} Secondly, to explore our hypothesis according to which WAD symptoms may be induced by a mismatch in the midbrain, periaqueductal gray (PAG) and adjoining regions, between the ascending information from cervical structures via the upper cervical cord to the mesencephalon, and the information from the vestibular and visual systems.\textsuperscript{18} And finally, to test if non-painful stimuli in the neck region of WAD patients was altered due to an ongoing process of central hyperexcitability.\textsuperscript{19}

\section*{Methods}

\subsection*{Participants}

Twelve WAD patients (grade one or two,\textsuperscript{5,20} between 2 and 10 years after the accident) and eight healthy volunteers, all females, were recruited via advertisement in public buildings, local newspapers and radio, national association of whiplash patients (Whiplash Stichting Nederland), and the Royal Dutch Society for Physical Therapy. Screening procedures included telephone questioning and brief interview. If the volunteer was considered electable, a neurological interview was scheduled for a final screening. Specific exclusion criteria for patients were loss of consciousness at the accident, neurological symptoms, and pain not related to WAD. Exclusion criteria for both WAD patients and healthy volunteers were current depression, anxiety, or other psychiatric disorder, organic brain disorder, somatic disease, left-handedness, substance abuse, pregnancy, and body mass index (BMI) $\geq 30$ kg/m$^2$.

All participants were asked to refrain from analgesics and anti-inflammatory drugs one to three days prior to scanning; from tobacco, alcohol and caffeine 12 h before, and from food 3 h before the PET scan. According to the Declaration of Helsinki, all participants gave written informed consent. The internal ethics committee of the University Medical Center of Groningen approved the experimental.

\subsection*{PET procedures}

During the whole procedure, the room lights were maintained at low intensity. The skin of the back part of the neck was rinse with alcohol, and disposable auto-adhesive skin electrodes (Model ST5090 5×9 cm, Axelgaard Manufacturing Co., USA) were placed at both sides of the neck, approximately at the level of the second and sixth cervical spinous process.

Four different conditions were used during the experiment, which were repeated three times resulting in a total of twelve scans per subject:

1. Rest state: no stimulation.
2. Low stimulation: 15% above the perception threshold.

3. High stimulation: 15% below observable muscular contraction or pain threshold.

4. Placebo-like stimulation: the participant was informed that will receive an electrical stimulation, below her perception level; but the device did not deliver any current.

The order of the sequences was modified between participants, keeping constant the rest scan as the 1st, 5th, and 9th scan. The stimulation consisted on a non-painful electrical constant current, delivered with the Digitimer DS7A device in combination with the stimulator DG2A (Digitimer Limited, UK), using a biphasic pulse, train repetition of 50 Hz, and 100 µs pulse duration. These parameters were selected based on a pilot test performed in a small group of healthy volunteers independent from this study ($n$=5). These values were found to allow an acceptable current range (mA) to define the thresholds, without apparent discomfort in the participant. Before the first PET scan, with the participant lying in the scanner, individual thresholds were determined by slow increase of the mA. First, the participant was asked to notify the researcher when the current was clearly perceived (lower threshold, considered as 0%). Then, the highest threshold (100%) was set when the participant notifies that the current was painful or unpleasant, or the researcher detected a clear muscular contraction. This procedure was repeated three times, with an interval of 1–2 min between trials. The average value of these thresholds was calculated, and used during the scan to define the low (15%) and high (85%) stimulation conditions. Before injection of the radiotracer the participant was informed about the condition that was going to be delivered as ‘rest’, ‘low’ for the placebo, ‘medium’ for the low stimulation (15%), and ‘high’ for the higher stimulation (85%). The device was turned on for all the conditions, except for rest condition. Immediately after each scan, the device was turned off, and the subject was allowed to open the eyes.

Each of the twelve PET scans was made in 3D mode (63 planes; axial field of view of 15.5 cm) using an Ecat Exact HR+ camera (CTI/Siemens, USA) with a spatial resolution of 4–5 mm full width at half maximum in all three directions. For the scan, the radiotracer $[^{15}\text{O}]\text{H}_2\text{O}$ was used as a measure of regional cerebral blood flow (rCBF). Per scan, 500 MBq of activity dissolved in 32 mL of 0.9% saline were administered intravenously into the right forearm at 8 mL/sec. After injection of the radiotracer, data were collected for 120 sec. Consecutive scans were made with intervals of 10–15 min. The participants were asked to keep their eyes closed during the scanning period, and their head was maintained in position using a head-restraining adhesive
band. A scan specific calculated attenuation correction was performed to minimize inter-scan displacement induced variance. Data acquisition was reconstructed using a filtered back projection procedure, corrected for background radiation, and summed to obtain a final 120 sec rCBF image.

**Subjective ratings**

During the neurological interview performed previous to the scan, all participants completed the Hospital Anxiety and Depression Scale (HADS), rated levels of pain between 0 and 10 (none to worst imaginable) on visual analogue scale (VAS), and the Neck Disability Index (NDI). An in-house translation of the Whiplash disability questionnaire (WDQ) was also provided.

**Neuroimaging analysis**

PET images were processed using the statistical parametric mapping (SPM8) software (Wellcome Department of Cognitive Neurology, London, United Kingdom) in combination with the Sandwich Estimator v1.2.1 (SwE) toolbox to account for the repeated measures. Images were first aligned between acquisitions for each subject, and then normalized to the Montreal Neurological Institute’s (MNI) stereotactic template. Images were masked for the whole brain image and then smoothed using a 12 mm Gaussian kernel. PET data was statistically evaluated with a small sample adjustment ‘type C2’ and ‘approximate III’ estimation for the degrees of freedom. A factorial design was used with group, conditions, and their interactions as factors. Differences in global blood flow were corrected using the ANCOVA between-subject and within-subject method. Level of significance was set to $p<0.005$ uncorrected, with an extent threshold of 100 voxels. Anatomical localization of the results was aided by the Hammers atlas.

In the WAD group, to explore the association between the altered rCBF and the symptoms, the statistical significant clusters obtained from the voxel-based analysis in SPM were divided according to the Hammers atlas, creating independent anatomical regions. The mean rCBF value for each of these regions was extracted from the original images and corrected for the mean whole brain uptake. These values were analyzed together with the scores obtained from the self-questionnaires (VAS, WDQ, NDI and HADS) using IBM SPSS Statistics 22 (SPSS Inc. Chicago, The United States). The Generalized Estimating Equations (GEE) model was used to account for multiple measures per subject and brain regions (clusters), with an auto-regressive correlation matrix. The age and BMI were also included in the model as covariates. No correction for multiple comparisons was applied, and $p<0.05$ were considered statistically significant.
Results

General statistics

All the participants were females, with a mean (± SD) age of 28±8 years for the control group (n=8), and 33±7 years for the patient group (n=12). No statistical differences were found between groups in age (p=0.15) or BMI (p=0.40). WAD patients suffered the accident 5±2 years before the study, and scored statistically higher for all the subjective ratings compared with the healthy volunteers (p<0.001). When comparing the sensitivity threshold, the highest threshold of the WAD patients was 83% of the one in healthy subjects (p=0.033), but not differences was observed in the lowest threshold between groups. More details can be found in Table 1.

Table 1. Differences between patients and control volunteers

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=8)</th>
<th>Patients (n=12)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>28±8</td>
<td>33±7</td>
<td>0.150</td>
</tr>
<tr>
<td>BMI</td>
<td>25±3</td>
<td>24±3</td>
<td>0.401</td>
</tr>
<tr>
<td>VAS</td>
<td>0</td>
<td>6±2</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>WDQ</td>
<td>0</td>
<td>63±19</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>NDI</td>
<td>0</td>
<td>24±6</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>HADS</td>
<td>0</td>
<td>10±6</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Lowest Threshold</td>
<td>39±8</td>
<td>33±8</td>
<td>0.147</td>
</tr>
<tr>
<td>Highest Threshold</td>
<td>72±10</td>
<td>60±13</td>
<td>0.033*</td>
</tr>
</tbody>
</table>

Mean values (±SD). BMI: body mass index, VAS: pain measured with visual analog scale, HADS: Hospital Anxiety and Depression Scale, NDI: Neck Disability Index, WDQ: Whiplash Disability Questionnaire. *p<0.05

Regional cerebral blood flow

No statistically significant differences were found between any of the experimental conditions in the voxel-based analysis (i.e.: rest, placebo or electrical stimulation of the neck), when the groups were compared independently or combined. However, statistically significant differences in the rCBF were found in several brain regions of WAD patients when this group was compared with the healthy volunteers (Table 2, Figure 1 and Figure 2). The peak voxels with increased rCBF on WAD patients was found in the right superior parietal gyrus (Z-score=3.76, x,y,z=13, -58, 17); while the peaks of decreased rCBF were detected in the right insula (Z-score=4.28, x,y,z=35, 0, -19), left insula (Z-score=4.23, x,y,z=-35, 22, -7), and right thalamus (Z-score=3.64, x,y,z=5, -12, 3).
Table 2. Alterations of the regional cerebral blood flow of Whiplash-Associated disorder patients compared with healthy volunteers ($p<0.005$ uncorrected, extent threshold $k=100$ voxels)

<table>
<thead>
<tr>
<th>Voxels in cluster</th>
<th>Cluster Z-value</th>
<th>Peak Z-value</th>
<th>Peak x,y,z</th>
<th>Peak location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy$&lt;$WAD</td>
<td>116</td>
<td>2.96±0.30</td>
<td>3.76</td>
<td>13, -58, 17 Superior parietal gyrus right</td>
</tr>
<tr>
<td>Healthy$&gt;$WAD</td>
<td>152</td>
<td>3.06±0.40</td>
<td>4.28</td>
<td>35, 0, -19 Insula right</td>
</tr>
<tr>
<td></td>
<td>107</td>
<td>3.03±0.39</td>
<td>4.23</td>
<td>-33, 22, -7 Insula left</td>
</tr>
<tr>
<td></td>
<td>147</td>
<td>2.94±0.27</td>
<td>3.64</td>
<td>5, -12, 3 Thalamus right</td>
</tr>
</tbody>
</table>

Peak voxel coordinates are in MNI space. Anatomical location according to Hammer’s brain atlas.

Figure 1. SPM results of the voxel-based analysis of $[^{15}\text{O}]\text{H}_2\text{O}$ scans. The results shown regions with increased (left) and decreased (right) regional cerebral blood flow (rCBF) in Whiplash Associated Disorder patients as compared with healthy volunteers ($p<0.005$ uncorrected, extent threshold $k=100$ voxels)
Figure 2. Voxel-based analysis of $[^{15}O]H_2O$ scans. The results shown some of the most significant regions with decreased or increased regional cerebral blood flow (rCBF) in Whiplash Associated Disorder patients as compared with healthy volunteers ($p<0.005$ uncorrected, extent threshold $k=100$ voxels)

**Association between subjective ratings and altered rCBF**

Within the WAD group, there were significant positive correlations (Table 3) between age and BMI ($r=0.71$, $p=0.01$), NDI scores and VAS pain ratings ($r=0.70$, $p<0.010$), NDI and WDQ ($r=0.71$, $p=0.01$), HADS and WDQ ($r=0.83$, $p=0.001$), and HADS and NDI ($r=0.65$, $p=0.022$). The rCBF values obtained from the four statistically significant clusters found previously in the voxel-based analysis were included in the GEE model as the outcome variable. The subjective ratings were included in the model as predictors, with the age and the BMI as covariates (Table 4). Only a positive association between the NDI score and the rCBF values extracted from the clusters was found in the analysis ($B=0.004\pm0.001$, 95% CI [0.001; 0.006], $p<0.009$).
Discussion

The present study was designed with the purpose to address three main research questions. First, the validation of the results showing hypoperfusion in the posterior parietal, temporal and occipital cortex, reported previously by Otte et al.,9,10 and Linnman et al.11 Secondly, to evaluate our hypothesis according to which a dysfunction in midbrain structures is behind most of the symptoms found in chronic WAD patients, with the PAG acting as a key player. And finally to explore the ongoing process of central hyperexcitability in WAD patients, comparing rest state brain perfusion with the expected changes in rCBF consequence of a non-painful stimulation. In this context, the increased rCBF found in the right superior parietal gyrus of the WAD group as compared with the healthy volunteers, seems to support that the parietal cortex is some how involved in the symptoms related with the

Table 3. Pearson correlations within the WAD patient group

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Time</th>
<th>BMI</th>
<th>VAS</th>
<th>WDQ</th>
<th>NDI</th>
<th>HADS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1</td>
<td>0.056</td>
<td>0.707</td>
<td>-0.520</td>
<td>-0.406</td>
<td>-0.341</td>
<td>-0.148</td>
</tr>
<tr>
<td>Time</td>
<td>1</td>
<td>-0.448</td>
<td>-0.362</td>
<td>-0.241</td>
<td>-0.207</td>
<td>-0.369</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>1</td>
<td>-0.205</td>
<td>-0.321</td>
<td>-0.195</td>
<td>-0.099</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS</td>
<td>1</td>
<td>0.521</td>
<td>0.705</td>
<td>0.355</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WDQ</td>
<td>1</td>
<td>0.710</td>
<td>0.835</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDI</td>
<td>1</td>
<td>0.650</td>
<td>0.022</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI: body mass index, VAS: visual analogue scale, WDQ: Whiplash disability questionnaire, NDI: neck disability index, HADS: hospital anxiety and depression scale

Table 4. Association between subjective ratings and alterations in the regional cerebral blood flow (rCBF) of WAD patients

<table>
<thead>
<tr>
<th></th>
<th>B ± SE</th>
<th>Lower</th>
<th>Upper</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>1.056 ± 0.024</td>
<td>1.009; 1.102</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Visual Analogue Scale (VAS)</td>
<td>0.004 ± 0.005</td>
<td>-0.007; 0.014</td>
<td>0.477</td>
<td></td>
</tr>
<tr>
<td>Whiplash Disability Questionnaire (WDQ)</td>
<td>-0.001 ± 0.001</td>
<td>-0.003; 0.001</td>
<td>0.282</td>
<td></td>
</tr>
<tr>
<td>Neck Disability Index (NDI)</td>
<td>0.004 ± 0.001</td>
<td>0.001; 0.006</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Hospital Anxiety and Depression Scale (HADS)</td>
<td>0.003 ± 0.003</td>
<td>-0.003; 0.008</td>
<td>0.365</td>
<td></td>
</tr>
</tbody>
</table>

SE: Standard Error; CI: 95% confidence interval
chronic WAD patients. In addition, we consider that the decreased rCBF detected bilaterally in the insula and in the right thalamus of the WAD group support the hypothesis of a misbalance in the interoceptive sensory system, even when no alterations of the perfusion were detected in the PAG with the current study design. And finally, no differences were found in the regional brain perfusion as consequence of the different levels of electrical non-painful stimulation.

There is an increasing evidence for a process of central hyperexcitability in chronic WAD patients, resulting in widespread lowered pain thresholds. In our study, volunteers were exposed to four different experimental conditions: absence of stimulation, placebo stimuli, and low/high non-painful stimulation of the neck. The first condition, without any stimulation, was intended to replicate the experimental setup presented by Linnman et al. While the placebo condition was introduced to assess the presence of altered brain perfusion patterns in relation with “beliefs and expectations” of WAD patient. And finally, the use of two different intensities levels of the electrical current stimuli pursued the exploration of rCBF changes in a non-painful condition. Contrary to our prior expectations, no alterations were observed in the rCBF as consequence of the different stimulation conditions, in either healthy or WAD patients. The absence of changes in the rCBF in regions such as the primary and secondary somatosensory cortex may be a consequence of the discrepancies generated by the lack of individual MRI scans to perform a more accurate process of spatial normalization of the scans; or may just reflect that the non-painful stimulations used in the present study design were not sufficient to induce substantial alterations in the rCBF that could be detected by the PET camera. However, a statistically significant lower tolerance to the electrical stimulation of the neck was found in the WAD patients when compared with the healthy volunteers, achieving unpleasant or painful feelings at lowest intensities of electrical current. These results support the idea that chronic WAD patients had alterations of their sensitivity thresholds, which is in agreement with the results presented in a recent meta-analysis of central hyperexcitability in chronic WAD patients.

Furthermore, when the rCBF of WAD patients was compared with healthy volunteers, brain regions commonly involved in the process of acute painful stimuli such as the insular cortex and the thalamus were found to exhibit alteration in its perfusion. In this sense, it is of special interest the decrease in rCBF that was observed bilaterally in the insula. Although no changes in perfusion of WAD patients were reported previously in this region, the insula it is been recognized as the primary reception area for the interoceptive sensory information, and its critical for the process of emotional feelings. Moreover, in a recent study performed with the radioligand $^{[11]}$CGR205171,
a significant lower neurokinin 1 (NK1) receptor availability was found in the left insula of chronic WAD patients, as well as in the frontal and cingulate cortex, hippocampus, amygdala, and the PAG. The NK1 receptors are widely distributed throughout the brain, and are the primary receptor of the neuropeptide “substance P” that modulates pain.\textsuperscript{39}

The increased rCBF found in the right superior parietal gyrus of the females chronic WAD groups seems to support the relevance of this region in the pathophysiology of WAD, as this changes are similar to those previously reported by Otte et al.,\textsuperscript{9–10} and Linnman et al.,\textsuperscript{11} involving parietal, temporal and occipital cortex. However, their results showed a hypoperfusion in those regions, while in the present study an increased rCBF was observed. This discrepancy in the direction of the results may be consequence of an error in the calculation of the global mean uptake within SPM software (unpublished work). Moreover, alterations in the rCBF shown by Linnmann et al. in the parahippocampal region or the cingulate gyrus, were not replicated in this study. These difference can be explained also by the discrepancies that arise when the data is analyzed in the MNI space, as is the case of SPM, but the results are reported using the Tailarach space.\textsuperscript{40} When the MNI peak coordinates, originally reported as parahippocampal region (18 -44 8), are explored using the Hammer atlas, the closest regions identified was the superior parietal gyrus. Independently of the final label used to classify the anatomical location of this region, in the present study these coordinates were found to be closer to the increased rCBF in found in WAD patients (i.e. right superior parietal gyrus).

Standard imaging techniques such as radiography, CT and MRI seem to be inconclusive for the prognosis of the symptoms after a whiplash trauma,\textsuperscript{8} and most of the current efforts are directed to understand the role of the tissue damage,\textsuperscript{7} and the processes involved in the transition from acute to chronic WAD.\textsuperscript{41} However, not much is known about how it is that reasonable simple accidents, often at rear-end low speed collisions, can induce brain processes such as the central hyperexcitability found in chronic WAD patients. We had hypothesized\textsuperscript{18} that the missing key for the understanding of the underlying pathophysiology in WAD is the close interaction between neck and midbrain thorough the spino-PAG and spino-thalamic fibers originated from the C1-C3 spinal segments.\textsuperscript{42,43} These neurons are distributed throughout lamina VI-VIII, with a specific cluster of them located in the ventrolateral horn.\textsuperscript{44} In addition, the trigeminocervical complex, constituted by the trigeminal nucleus caudalis and C1-C2 dorsal horn, maintains projections to the PAG and is known to be involved in the development of migraine and headaches.\textsuperscript{45} All these ascending pathways converge in the neurons located at the PAG and adjoining regions, basic for the normal function of multiple processes. In
WAD patients, the PAG and adjoining regions have been shown to express lower NK1 receptors, and undergo gray matter changes associated with the development of headache. In the present study no alteration in the rCBF of the PAG were detected. Adequate registration of the brainstem structures in our study using the automated method implemented in SPM8 was most probably suboptimal, especially in the absence of individual MRI images to be used in a more accurate process. However, the alterations detected in other brain regions related with interoceptive and pain processing pathways are appealing (i.e. bilaterally in the insula, and right thalamus).

Moreover, it seems to be an association between the rCBF values and the severity of the condition assessed by the NDI scores. Although these results must be taken with caution due to the small number of subjects included in the study these findings are of interest in the design of future experiments.

As mentioned before, the present study posses some limitations in its design. The most important one is that current results are drawn from a relative small sample size, compose only by females. While this is an important factor when interpreting the results related with the association of subjective scorings and the rCBF at different brain locations, we must consider that it has a relative lower relevance in the results obtained in the group comparison of rCBF. Since no differences were found between the presented stimuli, the comparison between chronic WAD patients and healthy volunteers was performed with 12 repetitions of the $[^{15}O]$H$_2$O scan per subject. These repeated measurements are important to control for within-subject variability, and strength the results of the analysis thanks to the use of advanced statistical methods such as those implemented in the “sandwich estimator” toolbox. Finally, the absence of individual MRI acquisitions may have influence in the accuracy of the registration procedure, making more difficult the precise exploration of rCBF changes in some regions such as the brainstem.

**Conclusion**

WAD symptoms might be the result of a mismatch between the proprioceptive information from the cervical spinal cord and the information integrated in regions such as thalamus and insula. Further investigation of the functional brain alterations present in WAD patients must be performed for a better understanding of its pathophysiology.
References

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