Exposure to diesel exhaust induces changes in EEG in human volunteers

Björn Crüts1, Ludo van Etten1, Håkan Törnqvist2, Anders Blomberg2, Thomas Sandström2, Nicholas L Mills3 and Paul JA Borm*1

Address: 1Centre of Expertise in Life Sciences, Zuyd University, Heerlen, The Netherlands, 2Department of Respiratory Medicine and Allergy, University of Umeå, Sweden and 3Centre for Cardiovascular Sciences, The University of Edinburgh, UK

Email: Björn Crüts - b.cruts@home.nl; Ludo van Etten - l.van.etten@hszuyd.nl; Håkan Törnqvist - hakan.tornqvist@lung.umu.se; Anders Blomberg - anders.blomberg@lung.umu.se; Thomas Sandström - thomas.sandstrom@lung.umu.se; Nicholas L Mills - nick.mills@ed.ac.uk; Paul JA Borm* - p.borm@hszuyd.nl

* Corresponding author

Abstract

**Background:** Ambient particulate matter and nanoparticles have been shown to translocate to the brain, and potentially influence the central nervous system. No data are available whether this may lead to functional changes in the brain.

**Methods:** We exposed 10 human volunteers to dilute diesel exhaust (DE, 300 \(\mu g/m^3\)) as a model for ambient PM exposure and filtered air for one hour using a double blind randomized crossover design. Brain activity was monitored during and for one hour following each exposure using quantitative electroencephalography (QEEG) at 8 different sites on the scalp. The frequency spectrum of the EEG signals was used to calculate the median power frequency (MPF) and specific frequency bands of the QEEG.

**Results:** Our data demonstrate a significant increase in MPF in response to DE in the frontal cortex within 30 min into exposure. The increase in MPF is primarily caused by an increase in fast wave activity (\(\beta_2\)) and continues to rise during the 1 hour post-exposure interval.

**Conclusion:** This study is the first to show a functional effect of DE exposure in the human brain, indicating a general cortical stress response. Further studies are required to determine whether this effect is mediated by the nanoparticles in DE and to define the precise pathways involved.

Introduction

Several epidemiological studies have identified diesel exhaust as an important component in determining the adverse health effects of particulate matter (PM) air pollution [1,2]. The molecular toxicity of diesel exhaust [3], is suggested to include oxidative stress-mediated inflammation, through particle surface, polycyclic aromatic hydrocarbons and redox active metals. Inflammation is considered to be central to both the pulmonary and systemic adverse health effects of diesel through environmental PM exposure [4]. Over the past decades several, several studies have suggested that inhaled nanoparticles are able to translocate to the brain via the olfactory nerves [5,6], where they have been associated with inflammatory changes at sites of deposition [7,8]. Passage to the brain is of particular concern since nanoparticles are potent induc-
ers of oxidative stress [4,9] and the brain is very sensitive to damage caused by oxidative stress [10]. Oxidative stress has been implicated in the pathogenesis of neurodegenerative diseases such as Parkinson’s and Alzheimer’s disease and it is conceivable that the long-term effects of PM exposure might include a decrease in cognitive function [11]. Exposure to PM in an experimental mouse model resulted in widespread activation of pro-inflammatory cytokines in the brain [7].

First epidemiological evidence for a functional effect of PM on brain function was presented in recent study that suggests an association between black carbon levels in the environment and cognitive development of children [12]. As a first experimental step to investigate functional effects of PM and its ultrafine fraction, we studied the short-term changes in brain activity induced by exposure to diesel exhaust. We exposed human volunteers for one hour to diesel exhaust (DE, 300 µg/m³) and filtered air and followed their brain activity using quantitative electroencephalography (QEEG) at different sites of the scalp during and after one hour after exposure. The exposure protocol reflects a peak exposure that may occur in environmental and occupational exposure to diesel exhaust.

**Methods**

Ten subjects (all male, mean age: 26 years, range: 18–39 years; free of neurological or psychopathological impairments) were exposed to diesel exhaust and filtered air during one hour (sham condition) in a blinded randomized crossover design, separated by a period of two to four days. Prior to the start of each exposure, resting brain activity was measured using QEEG outside the exposure chamber during 3-minute eyes open (Table 1) and 3-minute eyes closed periods. After the 1 hour exposure, EEG measurements were continued for another hour. Participants gave written informed consent and the study was approved by the Ethics Committee of the University Hospital of Umeå. Diesel exhaust was produced by a Volvo Diesel engine (Volvo TD45, 4.5 L, 4 cylinders, 680 rpm) as described previously [13], leading to 1.2 × 10⁶ suspended particles/cm³ (300 µg/m³) and gaseous pollutant levels of nitrogen dioxide (NO₂, 1.6 ppm), Nitrogen oxide (NO), 4.5 ppm, carbon monoxide (CO), 7.5 ppm and total hydrocarbons of 4.3 ppm.

During the EEG measurement, subjects rested in silence and performed no exercise to avoid interference on the QEEG measurement. Subjects sat in an upright position during the exposures, and were allowed to read a book to avoid boredom. EEG was continuously recorded from 8 electrode sites on the scalp according to the international 10–20 system: frontal pole (Fp1, Fp2), frontal (F3, F4), central (C3, C4) and parietal (P3, P4) at a sample rate of 500 Hz. The frequency content of the filtered EEG signals was calculated using Fast Fourier Transform (FFT) of 15 seconds intervals. From these intervals the Median Power Frequency (MPF) and spectral bands of the EEG were calculated. A second more detailed analysis divided the power spectrum into distinct frequency bands, including delta (1–3.5 Hz), theta (3.5–7.5 Hz), alpha (7.5–12 Hz), beta1 (12–20 Hz) and beta2 (20–32 Hz) bands. Changes in MPF and spectral bands during and after exposure are first presented as time series. From these time series, the mean MPF and spectral bands per hour and amplitudes of the first and last 5 minutes per exposure or post-exposure interval were calculated. Significance levels of differences (P < 0.05) between the first and last 5 minutes were compared between diesel and sham conditions by applying paired Wilcoxon tests to group results.

**Results**

**Changes in Median Power Frequency (MPF)**
Baseline measurements of MPF in the exposure chamber did not differ from pre-exposure measurements obtained outside the exposure chamber (data not shown). This shows that there is no acute effect of being in the exposure chamber, such as the presence of a pungent smell remaining after diesel sessions. There were also no significant differences at any time in MPF between subjects during the first 5 minutes of sham or diesel exposure. From 30 minutes into exposure we observed a slow increase in MPF of all subjects within the exposure chamber which was stronger in diesel exhaust exposure as compared to filtered air (Fig. 1). The effect on MPF was first observed and most

<table>
<thead>
<tr>
<th>Nr</th>
<th>MPF at Fp1 (Hz)</th>
<th>MPF at C3 (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.7</td>
<td>6.4</td>
</tr>
<tr>
<td>2</td>
<td>7.1</td>
<td>8.7</td>
</tr>
<tr>
<td>3</td>
<td>7.8</td>
<td>7.4</td>
</tr>
<tr>
<td>4</td>
<td>8.3</td>
<td>8.7</td>
</tr>
<tr>
<td>5</td>
<td>9.2</td>
<td>9.1</td>
</tr>
<tr>
<td>6</td>
<td>7.1</td>
<td>8.9</td>
</tr>
<tr>
<td>7</td>
<td>15.3</td>
<td>12.7</td>
</tr>
<tr>
<td>8</td>
<td>10.2</td>
<td>7.4</td>
</tr>
<tr>
<td>9</td>
<td>7.5</td>
<td>7.3</td>
</tr>
<tr>
<td>10</td>
<td>6.7</td>
<td>8.9</td>
</tr>
</tbody>
</table>

**Mean** 8.5 9.0 8.2 10.1 9.9 9.8 8.2 9.7

**SD** 2.7 1.9 1.2 2.3 2.6 2.6 1.1 1.1

Table 1: Individual absolute values of the median power frequency (MPF) at frontal locations (Fp1) and central location (C3) at pre- and post-exposure measurements (3 min each).
pronounced at the frontal electrode sites Fp1, Fp2, F3, and F4 (Fig. 2) and the average MPF at Fp1 and Fp2 was significantly different at the end of the diesel exhaust exposure compared to the start of exposure and to the sham conditions (P < 0.05 for both, Table 1). Interestingly the effect further increased during the diesel post-exposure period, when subjects were removed from the exposure chamber. This led to a significant difference between the 3 minute pre- and post-exposure measurements of MPF at the start and end of the post-exposure period both frontal (Fp1) and at the central (C3) location (Table 1). In addition the change over the exposure intervals, calculated from the difference between the first and last 5 minutes into the chamber, was significantly different between sham and diesel exposure (Table 2). This again indicated that tasks or factors present in the exposure chamber are unlikely to play a role in this effect on EEG. In addition, during post-exposure the effect on MPF spread in the other frontal F3 and F4 electrode sites leading to significant differences between diesel and sham (Table 2). Although slower changes in MPF were observed at the central (C3, C4) and parietal (P3, P4) electrode sites during the diesel (Fig. 2), at the end of exposure the mean MPF at post-exposure measurement was also significantly different from pre-exposure (Table 1).

Analysis of EEG frequency bands

Subsequently we extended the data-analysis to determine the effects of diesel exhaust on specific frequency bands from the EEG signal. The increased MPF at frontal electrode sites (Fp1, Fp2, F3, F4), is largely explained by an effect on fast wave activity (20–32 Hz, also denominated as beta2 activity). During diesel exposure, beta2 activity increased significantly compared to sham exposure. A marked increase in the beta2 signal was observed at the end of the diesel exposure interval in 7 out of 10 subjects (Fig. 3). The resulting change remained throughout the entire post exposure period. However, no such change in beta2 activity was observed at sham exposure (Fig. 3). Similar changes in fast wave activity were not observed at central and parietal electrode sites. Time variations of all frequency bands were analyzed using short-term Fourier-transformation, which revealed large variations in fast wave activity, with the largest variations over time in beta2 values (data not shown). Beta1-activity (15–20 Hz) also increased during diesel exposure at the frontal cortex, but this rise did not reach statistical significance (data not shown). Delta, theta and alpha activity showed no significant alterations during the diesel and post exposure periods compared to sham conditions (Fig. 3).

Discussion

This is the first study to demonstrate functional changes in brain activity as a result of exposure to diesel exhaust (DE) in human subjects. Our data demonstrate a delayed response to DE in the frontal cortex, characterized by an increase of median power frequency (MPF) and fast wave activity (21–32 Hz) in the EEG. These findings suggest an increased activity of the left frontal cortex during and after DE, but may be mediated by a number of different pathways.

As the first study of its kind interpretation of these findings are limited by a number of factors. Perhaps the most important limitation of our study is the fact that exposure to diesel exhaust is a mixture of combustion derived nanoparticles (CDNP) and exhaust gases (hydrocarbons, CO, NOx). A direct effect of nanoparticles after or upon the translocation of the nanoparticles in DE may play a role, but no direct evidence is presented for this explanation. It is known that upon inhalation a large fraction of nanoparticles will deposit in the nasal cavity [14]. From there DEP, but also carrier constituents such as polycyclic aromatic hydrocarbons (PAHs) and redox metals in DE may migrate through the epithelium to the olfactory bulb [5,6,8] and cause mild inflammation and toxicity[7,8]. Uptake of MnO2 nanoparticles in the olfactory bulb of rats has been reported beyond 6 hours hours after exposure, although no earlier time or inflammatory markers were investigated [7,8]. Therefore we think it is unlikely that the effects induced by a toxic or inflammatory effect of diesel particles in the brain. In addition, gases such as CO, NOx, and hydrocarbons may have mediated changes in EEG through vagal reflexes in the airways.
carbon monoxide (CO) generated by the heme oxygenase system has been shown innumerable studies to play a role in cardiac and neurophysiologic responses, but little information is available whether these findings are also relevant in traffic or diesel exhaust exposure. Interestingly, diesel particles may activate a pro-inflammatory vaso-vagal reflex in rats which is reduced by atropine [15]. This suggests that diesel particles themselves stimulate vagal reflexes in the airways and may as such cause feedback to the brain. On the other hand no difference in heart rate changes during diesel exposure compared to sham exposure. Following the diesel exposure MPF continued to increase resulting in significant differences at the frontal polar and the frontal sites (Fp1, Fp2, F3, F4) compared to the post-sham exposure period.

Table 2: Mean absolute change in median power frequency (MPF) at different locations during different exposure conditions.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Electrode locations</th>
<th>Fp1</th>
<th>Fp2</th>
<th>F3</th>
<th>F4</th>
<th>C3</th>
<th>C4</th>
<th>P3</th>
<th>P4</th>
</tr>
</thead>
<tbody>
<tr>
<td>∆Diesel</td>
<td></td>
<td>0.41*</td>
<td>0.34*</td>
<td>0.40</td>
<td>0.54</td>
<td>0.12</td>
<td>0.18</td>
<td>0.21</td>
<td>0.24</td>
</tr>
<tr>
<td>∆Sham</td>
<td></td>
<td>0.10</td>
<td>0.12</td>
<td>0.34</td>
<td>0.50</td>
<td>0.23</td>
<td>0.13</td>
<td>0.17</td>
<td>0.25</td>
</tr>
<tr>
<td>∆Diesel post</td>
<td></td>
<td>0.46*</td>
<td>0.43*</td>
<td>0.41*</td>
<td>0.60*</td>
<td>0.23</td>
<td>0.20</td>
<td>0.23</td>
<td>0.30</td>
</tr>
<tr>
<td>∆Sham post</td>
<td></td>
<td>0.21</td>
<td>0.24</td>
<td>0.09</td>
<td>-0.20</td>
<td>0.18</td>
<td>0.15</td>
<td>0.21</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Mean changes in the median power frequency between the first and last 5 minutes of all conditions are shown per electrode localization. *Significant differences between diesel and sham exposures, Wilcoxon paired test (P < 0.05).
slow-wave activity within a few minutes of onset of expo-
tered air exposure. Finally, olfactory sensitization to
alternating use of the exposure chamber for diesel and fil-
arious limitations, since neurochemical work and/or taking
samples of spinal fluid are not possible. Subjecting the
subjects to non-invasive techniques for imaging (Mag-
neurological work [12] is simply not possible at this
stage.

Ambient PM exposure has been associated with inflam-
mation in the lung, exaggerated airways responses and
increased morbidity as well as mortality in respiratory
and cardiovascular diseases [1,2]. Exposure to diesel exhaust
has been associated with similar effects, and has been
used as a surrogate exposure in many experimental studies
[3,13,15]. The observed effects of diesel exhaust on the
EEG add up to the evidence that air pollution may exert its
effects by a variety of pathways and may induce effects in
the brain. Based on the onset, location, frequency profile
and persistence of the response in EEG, we suggest that
our findings are due to an effect of nanoparticles that
slowly penetrate the brain or affect neurophysiologic sig-
neurochemical work and/or taking
samples of spinal fluid are not possible. Subjecting the
subjects to non-invasive techniques for imaging (Mag-

Interpretation of our findings is hampered by the lack of
similar studies on such a time-scale. Usually EEG studies
are done on short-term exposure or tasks, that last only a
few minutes. Therefore we have to consider that the
increased activity of the frontal cortex could also relate to
lowered vigilance of the subjects, caused by weariness or
getting bored during the exposure sessions. Indeed,
increased β2 values have been observed in combination
with lowered vigilance during cognitive tasks [17] and are
often regarded as compensation for lowered vigilance.
However this is usually associated with increased levels of
EEG slow wave activity [18], which was not observed in
this study. In addition, no significant effect of β2 fast-wave
activity was seen in the same subjects during sham expo-
ure. Therefore we conclude that decreased vigilance is
unlikely to explain the observed effects. Other studies
using EEG have linked frontal brain activity to various
physiological and psychological findings. Increased β2
levels have been reported in patients with neurological
and psycho-pathological disorders, such as headache,
post-traumatic stress disorder, burnout and traumatic
brain injury, and are often regarded as indicators of corti-
Figures 3

Figure 3
Absolute beta2 amplitudes (A) and theta amplitudes (B) at the left frontal cortex (F3). Changes are repre-
sented as a function of time in a superposed epoch analysis
graph for all subjects combined. Absolute power per fre-
quency band is visualized for diesel (blue line) and sham con-
dition (black line), combining the one hour exposure
condition and subsequent post condition in one graph. Both
lines represent a 5 minute moving average of the original sig-
nal. Beta2 (A) but not theta-amplitudes (B) are elevated dur-
ing diesel exposure compared to sham exposure.

was seen between exposures, which would have occurred
of the current diesel exhaust had induced a vagal reflex.
A further complication is that the exposure room was con-
taminated with a pungent smell and it cannot be excluded
that the smell of diesel exhaust plays a role in the
increased cortical arousal in the exposed subjects. How-
ever a confounding effect on our findings by the smell is
unlikely for several reasons. First, subjects reported only
discomfort due to smell in the first minutes while changes
in EEG were only seen at the end of the exposure interval.
Secondly, the effects on MPF continued to rise after sub-
jects left the exposure chamber. Thirdly, the diesel odor
was also present during the sham condition, because of
alternating use of the exposure chamber for diesel and fil-
tered air exposure. Finally, olfactory sensitization to
chemical stimulation is usually noted as an increase of
slow-wave activity within a few minutes of onset of expo-
sure [16]. This slow wave activity was unaffected during
the entire study.

Features Additional Information
netic Resonance Imaging, MRI) or measuring (Near Infra-red Resonance Spectroscopy, NIRS) blood flow and tissue damage are the options that are currently being explored to follow-up these human studies. Current work concentrates on the similar effects of artificially generated nanoparticles that are not contaminated with gases and other components.

**Authors’ contributions**

BC and LE carried out the pilot-measurements and data processing of the EEG signals, and prepared and supervised the project on-site; HT, was responsible for the exposure assessment, AB and NM were the responsible medical doctors for the survey; TS and PB were the coordinators and planners of the study. The manuscript was written by BC and PB, but all authors read, corrected and approved the manuscript.

**Acknowledgements**

The authors would like to acknowledge Maastricht Instruments (Maastrcht, NL) for providing the MPAQ system; Biometrisch Centrum (Gulpen, the Netherlands) for providing the QEEG, Annika Johansson and Helena Bogseth (research nurses at University Hospital, Umeå, Sweden), and finally SKO, the British Heart Foundation (RG/05/ 003), the Swedish Lung-HeartFoundation and the Swedish National Air Pollution programme for supporting this research effort.

**References**


---

**Publish with BioMed Central and every scientist can read your work free of charge**

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."  
Sir Paul Nurse, Cancer Research UK

Your research papers will be:

* available free of charge to the entire biomedical community
* peer reviewed and published immediately upon acceptance
* cited in PubMed and archived on PubMed Central
* yours — you keep the copyright

http://www.biomedcentral.com/info/publishing_adv.asp