Final Progress Report

SOLVENT-RELATED FUNCTIONAL BRAIN ABNORMALITIES (OH003646; 9/30/01 – 09/29/05)

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Abstract

This study investigated central nervous system (CNS) integrity and neuropsychiatric status in a sample of workers with past exposure to solvents while employed in the Railroad (RR) industry. Solvent exposure was evaluated with a structured clinical interview to ascertain an estimate of cumulative lifetime exposure to solvents in the workplace. Exposed workers and a matched group of non-exposed blue collar workers underwent magnetic resonance imaging (MRI) to assess brain structure (e.g., white matter) and positron emission tomography (PET) to asses brain function. A battery of neuropsychological tests was also administered, as well as indices to assess psychiatric status. Exposed workers were selected from a sample of over 250 workers who had been seen in the Occupational and Environmental Health Clinic at the West Virginia University School of Medicine between 1995 and 2003. The subjects in the exposed group had been employed in the RR industry and had been exposed to solvents for at least 10 years. Estimates of lifetime exposure were based on years of exposure, symptoms during exposure (e.g., fresh air breaks), use of protective equipment, route of exposure (e.g., airborne, dermal), etc. Demographically matched non-exposed blue-collar control workers were recruited from the surrounding community and completed the same battery of tests and compared to exposed subjects.

Our specific aims were to: 1) Determine, as measured by [O^{15}] water PET methodology, brain regions which are associated with performance of cognitive tasks of working memory and executive function. 2) Contrast the unique aspects of brain structure and brain function of solvent-exposed subjects to demographically matched, non-exposed control subjects. 3) Assess neuropsychiatric status in all subjects with a battery of cognitive and psychiatric tests. 4) Analyze CNS integrity as a function of neuropsychological performance, psychiatric status, and exposure.

Study findings to date show that the volume of the corpus callosum – an area with large white matter fibers – as measured by MRI, was reduced in subjects with solvent exposure and the volume of the corpus callosum was related to the extent of exposure and neuropsychological performance. Specifically, greater exposure was associated with smaller corpus callosum volume and smaller corpus callosum volume was associated with poorer performance on cognitive tests of executive function. We have also found that the volume of the prefrontal grey matter is reduced in subjects with solvent exposure and the volume is inversely related to extent of exposure and positively related to executive functions. In terms of the PET data, solvent exposed subjects show greater activation in the prefrontal cortex when performing cognitive tasks, relative to normal controls. The extent of activation is associated with extent of exposure such that increased exposure results in increased activation in the frontal cortex to perform the tasks. With regards to cognitive function, we found that exposed workers performed more poorly than non-exposed controls across several cognitive domains (e.g., memory and learning, executive function) and cognitive decrements in the exposed workers were significantly predicted by their overall estimate of lifetime exposure to solvents.

Highlights/Significant Findings

Findings from this study indicate CNS alterations in solvent-exposed workers compared to matched controls. Initial data analysis was conducted on brain white matter via MRI. The volume of the corpus callosum was determined for exposed and non-exposed subjects and significant decreases were noted in the exposed group in the genu of the corpus callosum. Moreover, smaller volume in the corpus callosum was associated with greater lifetime solvent exposure and poorer performance on cognitive test scores. These results have recently been accepted for publication (Haut et al., 2005). In a second analysis on brain volume, we measured the amount of prefrontal grey matter via MRI. The volume of the prefrontal grey matter was decreased in the exposed group relative to the non-exposed group. Moreover, smaller volume of the prefrontal grey matter was associated with greater lifetime solvent exposure and poorer performance on test of executive function. This finding is being prepared as a manuscript. A second wave of analyses was conducted on the cognitive and psychiatric indices. Preliminary data analyses show that exposed subjects have lower performance across several cognitive domains compared to matched non-exposed controls and performance on the cognitive tests (particularly tests of executive function, memory, and spatial ability) are predicted by the lifetime solvent exposure index. While exposed subjects have higher psychiatric symptoms (i.e., depression), this is not predictive of cognitive status. Finally, we are currently analyzing activation data from the PET scan procedures. From the findings to date, we have observed that the exposed subjects activate more prefrontal cortex than non-exposed subjects on a working memory task, a recognition memory task, and a frequency memory task, which is similar to working memory. In addition, for each of these tasks greater lifetime exposure is associated with greater activation in the prefrontal cortex to complete the tasks. The analyses of the PET data as a function of neuropsychological performance are in the final stages. We have found no difference between solvent exposed subjects who have psychiatric symptoms and those who do not have psychiatric symptoms for MRI measures of brain volume in the corpus callosum and prefrontal white matter. Results of the PET analysis comparing solvent exposed subjects with and without psychiatric symptoms are being completed.

Translation of Findings

This research has important implications for workplace solvent exposure. The findings suggest that higher lifetime exposure to organic solvents may result in loss of brain white matter and grey matter as well as decrements in cognitive function. Every effort should be made to provide protective equipment for workers in occupational settings where solvent exposure is common. These data point to the risk for alterations in brain white matter and grey matter and development of neuropsychiatric disorders in workers with long-term exposure to organic solvents in an occupational setting. While there are currently permissible exposure for exposure to organic solvents, data from other studies and from this project lend support to the belief that care should be taken in occupational settings to minimize solvent exposure. Moreover, physicians should be

made aware that workers with past exposure to solvents in the workplace may be at risk for changes in brain structure, brain function and cognition.

The demonstration of an association between lifetime solvent exposure and central nervous system disorder may have practical applications as well. Over 1 million workers continue to be employed in solvent-handling trades. The application of more sophisticated epidemiological designs and more sensitive measures of outcome and exposure should be addressed to determine more exact levels for permissible exposure limits and at what point decrements may occur. While the overwhelming majority of studies assessing solvents and CNS and cognitive status indicate significant alterations for exposed workers, a few researchers continue to suggest that solvents may have little effect on the CNS. The current findings dispute this and indicate that, at least for this cohort, solvents can result in significant loss of brain white matter and cognitive decrements. Future studies should address whether these change pose riskier prospects for aging workers. That is, does occupational exposure to solvents in earlier decades put one at greater risk for development or onset of age-related neurological degenerative diseases (e.g., Alzheimer's disease)?

Scientific Report

Solvent exposed participants were selected from clinic referred RR workers (seen at the Occupational and Environmental Health Clinic at the West Virginia University School of Medicine) who reported both significant exposures in their occupational environment and complaints of cognitive dysfunction (e.g., memory and/or concentration problems) between 1995 and 2003. To be considered for the study, a minimum of 10 years of solvent exposure was required. *Exclusion criteria* included pending or ongoing litigation regarding exposure, current substance abuse or illicit drug use, history of neurologic or neurosurgical condition (e.g., stroke, closed head injury, brain tumor), history of psychiatric problems prior to exposure or history of other serious medical illness (e.g., myocardial infarction, cardiac surgery, organ failure). Subjects with medically controlled conditions such as hypertension or non-insulin dependent diabetes were included. A total of 258 patient files were reviewed. Those patients that passed an initial screening based on historical information contained in the files were then contacted by telephone (n=113). Of these, 37 patients agreed to participate in the study, 57 declined to participate, and the remaining 19 patients were excluded based on the above exclusion criteria.

An equal number of non-exposed control subjects were recruited from community locations and were matched to the exposed subjects in terms of age, education, similar blue-collar occupational and socioeconomic status. The control subjects included maintenance workers at West Virginia University, trade unions in the region, and others who responded to newspaper advertisements. Control subjects were not considered if they reported any solvent exposure in their occupation (e.g., painters) or through other means (e.g., accidental exposure, hobbies). All potential control participants underwent the medical screening described above over the telephone and were subjected to the same exclusion criteria as the solvent exposed subjects. All partici-

pants were Caucasian males. Prior to participation, all subjects signed an informed consent form approved by the Institutional Review Board at West Virginia University and the University of Pittsburgh.

Solvent exposure interview. Each solvent exposed subject underwent a structured interview to assess exposure. The exposed subjects had worked at two principal sites and described uncontrolled, long-term, intense skin contact and inhalation exposures to a variety of cleaning solvents and solvent mixtures, including tetrachloroethylene, trichlorethylene, trichloroethane, and mineral spirits. Settings and job duties included locomotive degreasing operations that applied 55-gallon drums of solvents through a spray wand, entry into enclosed locomotive spaces still wet with evaporating solvent, part and tool clean-up operations involving inhalation and frequent skin contact, and large surface applications for degreasing and clean-up purposes. For most of their work histories, workers described multiple episodes of acute neurointoxication symptoms including headache, nausea, and a "giddy-headed" sensation, frequent permissible "fresh air" breaks to address symptoms, absence of respirator use, and intermittent or no use of protection from dermal exposure. The average number of years of exposure was 24.3, with a range of 10-35 years. The average length of time between termination of exposure and evaluation was 8.44 years (range 1-24) and their most recent exposure was greater than one year prior to enrolling in the study.

Neuropsychological Assessment and Psychiatric Interview. The Pittsburgh Occupational Exposures Test (POET) Battery was administered, as well as additional tests of cognitive function. The POET measures a range of cognitive skills (general intelligence, learning and memory, visuospatial, attention, perceptuomotor efficiency, and speed and dexterity) and has been shown to discriminate solvent-exposed persons from demographically similar controls. Included are subtests from the Wechsler Adult Intelligence Scale and the Wechsler Memory Scale, and the Halstead-Reitan Battery. Additional tests were included to supplement the POET, and included more complex tests of working memory, executive function and problem solving. In addition to the cognitive testing, all subjects completed a comprehensive structured psychiatric interview, the Structured Clinical Interview for DSM-IV (SCID-IV), as well as the Beck Depression Inventory.

The Specific Aims are those that we initially described in application were:

Specific aims:

1. Determine, as measured by [^{15}O] water PET methodology, brain regions which are associated with performance of cognitive tasks of working memory and executive function.
2. Contrast the unique aspects of brain structure and neural activation of solvent-exposed subjects to demographically matched, nonexposed control subjects.
3. Assess neuropsychiatric status in all subjects with a battery of cognitive and psychiatric tests.
4. Analyze neural activation as a function of neuropsychological performance, psychiatric status, and exposure index assessment.

Preliminary Results:

Magnetic Resonance Imaging and Determination of White Matter and Grey Matter Volumes: To date we have completed analysis of brain volumes between the solvent exposed group and the normal control group. We have measured the volume of the corpus callosum in all subjects who received an MRI scan (31 per group). Some subjects could not receive an MRI scan because of metal in the body or they were too big to fit in the scanner. Data was not usable from 2 control subjects. The raw volume of the total corpus callosum was significantly lower in exposed participants (M = 6.8) compared to controls (M = 7.5), $F(1,61)$= 6.51, $p < .05$. The anterior segment of the corpus callosum was significantly different between the two groups, with a smaller volume in the exposed participants (M = 2.9) compared to the controls (M = 3.4), $F(1,61)$= 3.5, $p < .05$. Expressed as a percentage of total supratentorial intracranial volume, the total corpus callosum was significantly smaller in exposed participants (M = .67%) compared to controls (M = .74%), $F(1,61)$= 6.12, $p < .05$. In addition, expressed as a percentage of the total supratentorial intracranial volume, the anterior segment of the corpus callosum was smaller size in subjects with exposure (M = 0.29%) compared to the controls (M = 0.34%), $F(1,61)$= 9.49, $p < .05$. No other region of the corpus callosum showed a significant group effect. Exposure classification was negatively related to the total volume of the corpus callosum, $r = -.41$, $p < .05$ and to the volume of the anterior segment, $r = -.39$, $p < .05$, indicating smaller volumes were associated with greater exposures. These results held when examining a subset of subjects who did not have confounding medical conditions and in this subgroup we also observed significant correlations between corpus callosum volume and cognitive performance; Letter-Number Sequencing was significantly correlated with anterior corpus callosum volume ($r = .450$, $p < .05$) such that worse performance was associated with smaller volume. There was a significant inverse correlation between Stroop Color-Word performance and anterior corpus callosum volume ($r = -.385$, $p < .05$), such that smaller volume was associated with more interference (i.e., worse performance). There was a trend for a relationship with Part B of the Trail Making Test ($r = -.254$, $p = .06$) such that smaller volume was associated with slower performance. There was a weak trend with verbal fluency, such that smaller volume was associated with worse performance, ($r = .207$, $P = .10$). There was no difference in corpus callosum volume between the exposed subjects with versus without a concurrent psychiatric diagnosis. These findings have been accepted for publication (Haut et al., 2005). A copy of the manuscript is included with this progress report.

We have also completed analysis of prefrontal cortex grey matter volumes using MRI. We have compared the 19 solvent exposed subjects without confounding medical conditions (e.g., hypertension, diabetes) to 19 of the age and education matched normal control subjects. A one-way analysis of variance revealed that there was a trend for a difference between the groups for the raw prefrontal lobe volume, $F(1,36)=3.41$, $p = .07$ with the individuals with exposure having a smaller volume. When correcting for total supratentorial intracranial volume, the individuals with exposure have a smaller volume of the prefrontal grey matter, $F(1,36)=5.06$, $p < .05$. The corrected volume was negatively correlated with exposure, $r = .37$, $p = .05$, such that increased history of exposure

was associated with decreased volume of the prefrontal grey matter. Finally, the corrected volume of prefrontal cortex was positively correlated with performance on the Stroop Color Word Test – color word subtest, $r = .52$, $p < .05$ such that in the exposed subjects smaller prefrontal volume was associated with worse performance. The correlations between prefrontal volume and the other neuropsychological measures were not significant. These results are being written up as a manuscript at the present time.

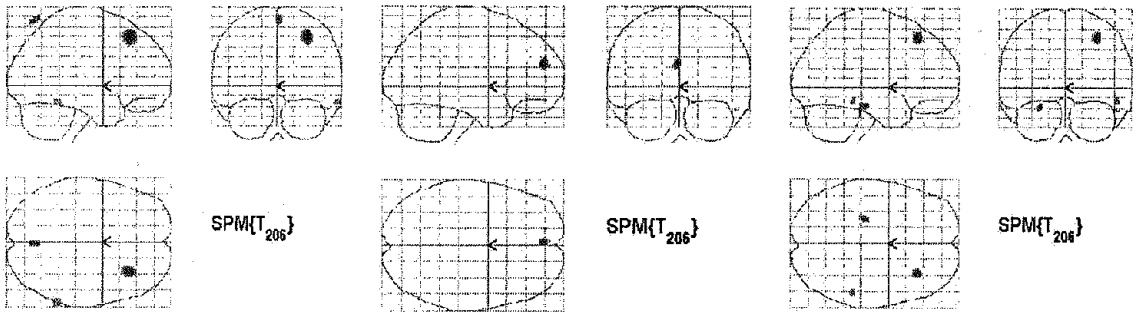
Positron Emission Tomography and Working Memory/.Executive Tasks: We have completed the primary analysis of the PET data. We used 3 activation tasks. 1) working memory, 2) recognition memory, 3) frequency memory. Each of these tasks was compared to a control or subtraction condition and we also completed 4) a resting scan on each subject.

1) *Working memory*: This task involved having subjects count from 1 – 10 in a different order for 60". The subtraction task was counting from 1 – 10 over and over in order for 60". When examining the activation associated with this task in normal controls subjects we observed robust activation in the parietal cortex and the frontal cortex bilaterally. This is highly consistent with the activation typically observed for working memory tasks. When we compared the activation in the solvent exposed group to the non-exposed group, there were no differences. However, the task was performed differently between groups and the solvent exposed group also had more medical confounds than the non-exposed group. Therefore we compared only those subjects without medical confounds and covaried behavioral performance on the task. The results of this analysis revealed the exposed group had an area of significantly greater activation in the right prefrontal cortex relative to the non-exposed group.

2) *Recognition Memory*: This task involved having subjects listen to a list of words prior to scanning and then during scanning respond yes or no to a whether a word had been presented prior to scanning. The control subtraction task was to listen to a word and say the number 3. Activation in the control subjects demonstrated regions of activation in the frontal cortex bilaterally. Compared to the exposed group, there where minimal differences in activation. As with 1) above we excluded the medically confounded subjects and co-varied behavioral performance during the scanning. Exposed subjects demonstrated significantly greater activation in the left prefrontal cortex and the non-exposed subjects demonstrated significantly greater activation in the left parahippocampal gyrus.

3) *Frequency Memory*: This task involved having subjects listen to a list of words prior to the scan. The words repeated between 1 – 6 times. During the scan, subjects were required to say a number 1 – 6 to indicate the number of time they heard the word. The same control/subtraction condition that was used in 2) above was used in this condition. Activation in the control subjects demonstrated regions of activation in the frontal cortex bilaterally. There were three small areas of differences between groups in the frontal cortex (2 solvent > control; 1 control > solvent). After controlling for behavioral performance and medical confounds, there was one strong area of dif-

ference in the right prefrontal cortex, with exposed subjects showing greater activation than controls. Below are the activation maps for working memory (left), recognition memory (center) and frequency memory (right), showing activation areas that are greater in prefrontal cortex in exposed subjects compared to nonexposed subjects.



Summary of Activation studies: For each of the tasks, the exposed group demonstrated greater activation of the prefrontal cortex than the non-exposed group. These results, along with the findings on structural imaging outlined above, suggest that exposed subjects have underlying frontal lobe impairments, and thus require additional activation to carry out cognitive tasks sub served by the frontal lobes.

4) For the resting condition (eyes closed and resting for 60"), the exposed group had decreased activation in the medial prefrontal cortex and the thalamus. When controlling for the psychiatric status of the patients and the medical confounds these areas of difference remained.

Remaining areas of analysis: 1) comparison of exposed subjects with psychiatric symptoms to exposed subjects without psychiatric symptoms for each of the 3 activation tasks. 2) correlation between the neuropsychological domains and each of the 3 activation tasks (e.g., are there areas of the brain where amount of activation during working memory that correlate with the five cognitive domains listed below).

Neuropsychological Assessment: For the current assessment, solvent exposed subjects and non-exposed controls do not differ in age or education. As noted above, the POET battery was administered along with additional tests of working memory, executive function and problem solving. A multivariate analysis of variance was done to compare solvent exposed and non-exposed subjects. The solvent-exposed subjects had significantly lower scores on the majority of cognitive tests. Statistically significant group differences were found for the cluster of executive tests [$F = 3.89$, $p < .05$], and nearly significant levels were noted for tests of learning and memory [$F = 3.63$, $p < .06$]. Within group multiple regression analysis was done to assess neuropsychological outcome and exposure across the five cognitive domains: learning and memory, executive function, spatial ability, motor speed, general intelligence. Age and education were entered in the first block, followed by scores on the Beck Depression Inventory and a measure of pre-morbid IQ entered on the second block. The lifetime exposure index was entered in the final step. Not surprisingly, pre-morbid estimates of IQ accounted

for a significant amount of the variance for most tests. However, estimated lifetime exposure to solvents accounted for a significant portion of the variance on the learning and memory (R^2 change $p < .04$), executive function (R^2 change $p < .02$) and spatial ability (R^2 change $p < .02$) domains. These data analysis are on-going and a draft paper is in the initial stages.

Inclusion of Gender and Minority Study Subjects

The aim of the current study was to determine brain changes and cognitive function in a sample of railroad workers with prior exposure to lead in the workplace. Therefore, testing was only conducted on these workers (all were men) and no women or children were assessed in this project.

Related Publications

Haut MW, Kuwabara H, Ducatmen A., Hatfiled G, Parsons MW, Scott A, Parsons E, Morrow LA: [2005]. Corpus Callosum Volume in Railroad Workers with Chronic Exposure to Solvents, *Journal of Occupational and Environmental Medicien, in press.*

Haut, M., Kuwabara, H., Morrow, L., Hatfield, G., Parsons, M., Scott, A. & Ducatman, A. Solvent-related neuroimaging changes in railroad workers. Presentation to the International Neuropsychological Society. St. Louis, 2005.

Morrow, L., Haut, M., Ducatman, A., Parsons, E., Parson, M., Metheny, K. & Scott, A. Neuropsychological deficits in railroad workers with long-term solvent exposure. Presentation to the International Neuropsychological Society. St. Louis, 2005.

Materials Available for Other Investigators

Not applicable.