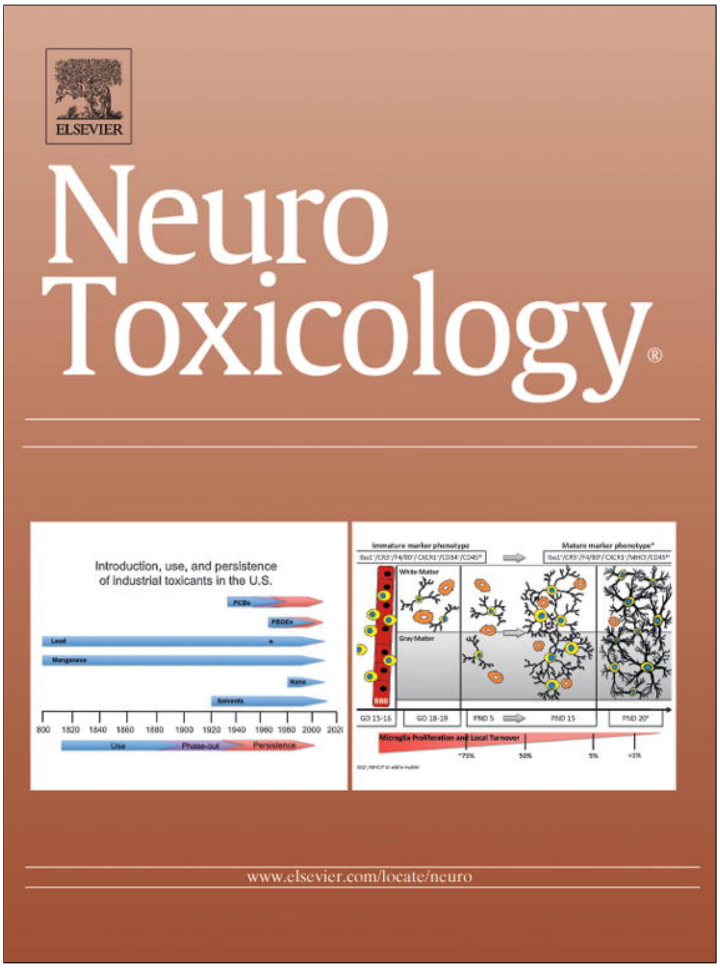
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# Vanadium exposure-induced neurobehavioral alterations among Chinese workers

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ABSTRACT

Vanadium-containing products are manufactured and widely used in the modern industry. Yet the neurobehavioral toxicity due to occupational exposure to vanadium remained elusive. This cross-sectional study was designed to examine the neurotoxic effects of occupational vanadium exposure. A total of 463 vanadium-exposed workers (exposed group) and 251 non-exposed workers (control group) were recruited from a Steel and Iron Group in Sichuan, China. A WHO-recommended neurobehavioral core test battery (NCTB) and event-related auditory evoked potentials test (P300) were used to assess the neurobehavioral functions of all study subjects. A general linear model was used to compare outcome scores between the two groups while controlling for possible confounders. The exposed group showed a statistically significant neurobehavioral alteration more than the control group in the NCTB tests. The exposed workers also exhibited an increased anger-hostility, depression-dejection and fatigue-inertia on the profile of mood states ($p < 0.05$). Performances in the simple reaction time, digit span, benton visual retention and pursuit aiming were also poorer among exposed workers as compared to unexposed control workers ($p < 0.05$). Some of these poor performances in tests were also significantly related to workers' exposure duration. P300 latencies were longer in the exposed group than in the control ($p < 0.05$). Longer mean reaction times and more counting errors were also found in the exposed workers ($p < 0.05$). Given the findings of our study and the limitations of neurobehavioral workplace testing, we found evidence of altered neurobehavioral outcomes by occupational exposure to vanadium.



## 1. Introduction

Vanadium is widely distributed in the earth's crust and is a major trace metal found in fossil fuels such as oil, coal and shale. Vanadium compounds are extensively used in the production of metal alloys and sulfuric acid (as a catalyst), in petroleum and chemical engineering, and in welding. More than 90% of industrial vanadium is used for making steel. The market demand for vanadium over the past three years has shown a continued increase worldwide demand, most notably in China, for its higher strength in vanadium steel (Bunting, 2006). Extensive industrial application has led to vanadium-related occupational exposure. In addition, the combustion of fossil fuels containing vanadium results in a significant environmental exposure to vanadium.

Earlier studies have shown that vanadium exposure may cause respiratory dysfunction (Woodin et al., 2000; Todaro, 1991; Musk and Tees, 1982), hematologic and biochemical alterations, renal toxicity (Al-Bayati et al., 1989; Zaporowska and Waailewski, 1992; Jandhyala and Horn, 1983), reproductive and developmental toxicity (Lahav et al., 1986), immunotoxicity, mutagenicity (Chakraborty et al., 2000), and neurotoxicity (Sanchez et al., 1998; Poggioli et al., 2001; Avila-Costa et al., 2004). The cases of death due to exposure to vanadium compounds have also been reported in literature (Sjoberg, 1950). Most of reported vanadium toxicological studies are conducted in animal models; only relatively small numbers of studies are done on occupational workers. While some studies have shown vanadium-induced neurotoxicity, the question as to whether vanadium exposure causes neurobehavioral alterations, was unanswered. Since the central nervous system (CNS) is sensitive to vanadium toxicity, an in-depth study on vanadium-induced neurobehavioral changes, especially under chronic, low-level occupational exposure, became necessary.

The neurobehavioral core test battery (NCTB) is widely used testing method established by the World Health Organization

*Abbreviations:* NCTB, neurobehavioral core test battery; P300, event-related auditory evoked potentials test; CNS, central nervous system.

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(WHO) for early detection of CNS impairment caused by neurotoxic agents present in the working environment. It provides a rapid, stable and effective test for cognitive and neurobehavioral toxicology (Johnson, 1990; Anger et al., 2000; Anger, 2003). Event-related auditory evoked potential test (P300) has been used in clinics and laboratories to test psychological alterations for its unique association with certain cognitive functions such as the decision making processes, etc. (Soltani and Knight, 2000; Braverman and Chen, 2006). The purpose of this study was to test the hypothesis that occupational exposure to vanadium in a low-dose, long-term exposure condition may lead to the early onset of neurobehavioral changes among vanadium-exposed workers. The NCTB and auditory event-related potentials (P300) were used in assessing the neurobehavioral outcomes.

## 2. Materials and methods

### *2.1. Study population*

The participants were selected from a Steel and Iron Group in Panzhihua area, China. The manufacturer is located in the Sichuan Province, a mountainous region of south-western China. The factory has been in manufacture of vanadium-containing products since 1989 and currently has more than 4000 workers. Study subjects were recruited to the exposed and control groups based on their job classification. The exposed group comprised 463 workers who had worked in the vanadium-containing steel production workshop for at least 1 year, whereas the workers of the control group (251) were recruited from a workshop that belongs to the same Steel and Iron Corporation but produces the steel products without containing vanadium. The two factories were established around the same time; the vanadium products workshop has been in operation since 1989 and the cold rolling mill in the non-vanadium steel workshop was built in 1993. The workers in both workshops were trained by the same vocational training school. The cold rolling mill is located about 5 km (3.1 miles) away from the vanadium production workshop. The data by routine occupational safety monitoring of air vanadium level indicate that vanadium smoke time-weighted average concentration in the exposed group was 0.216 mg/m$^3$, while the value in the control group is 0.013 mg/m$^3$.

The workers in both workshops underwent the routine physical examination under the health surveillance for normal function of skin, mucous membranes, lung, heart, liver, kidneys and blood system. All study subjects in the current control and exposed groups have passed these exams and were considered the "healthy" workers. The definition, however, does not exclude the abnormality in the central nervous system, and/or the cardiovascular system, among others.

The mean ages were (39.5 $\pm$ 7.8) years (range: 20–60) and (37.1 $\pm$ 5.8) years (range: 20–60), respectively, for the vanadium-exposed group and controls. Both group workers had the similar socioeconomic status (salary, education, etc.) and background environmental factors (place of residence, etc.). Subjects in both groups at the time of interview had reported no exposure to other toxic substances, radiation therapy, or substance abuse. There were no statistically significant differences in smoking and alcohol consumption between the vanadium-exposed workers and the controls.

### *2.2. NCTB examination*

Participating subjects were invited to the West China School of Public Health Sichuan University. A written consent form was obtained from each subject prior to the onset of the study. The study protocol received official approvals from the Office of Clinical Investigation at Sichuan University, which is responsible for ethical and subject protection issues in the university. Medical examinations were conducted to rule out conditions related to central or peripheral nervous system illnesses, musculoskeletal problems or psychiatric disorders, such as epilepsy, Parkinson's disease, Alzheimer's disease, hyperthyroidism, and color blindness. None of the workers were found to have any major health issues at the time of tests. The neurobehavioral tests were conducted in the morning (8:00–12:00). The tests were conducted in a group setting. The POMS test, Santa Ana dexterity, digit symbol, Benton visual retention and pursuit aiming test were conducted in a big room with the curtains that separate participants. Simple reaction time and digital span were tested with the subject alone in an enclosed quiet room.

The NCTB includes a profile of mood states (POMS) questionnaire and a set of neurobehavioral tests. The POMS questionnaire consists of six mood scales: anger-hostility, confusion-bewilderment, depression-dejection, fatigue-inertia, tension-anxiety and vigor-activity. The participants were asked to fill out the questionnaire, which cover the basic demographic information (e.g., age and educational status), the information on smoking, alcohol consumption, medications, recent medical history, subjective symptoms, and the job description including type of job, duration of work, and total duration of vanadium-related work.

### *2.3. Neurobehavioral tests*

The neurobehavioral tests included: (1) simple reaction time, including two extreme values, fastest simple reaction time, slowest simple reaction time and mean simple reaction time; (2) digit symbol; (3) Santa Ana dexterity, including preferred hand and non-preferred hand; (4) digital span, including forward value and backward value; (5) Benton visual retention; and (6) pursuit aiming (PA), including total PA and correct PA. We used all of these tests to assess the participants' neurobehavioral reaction or status.

*Simple reaction time*. Simple reaction time was assessed using the standard reaction time tester (Model M40305, Midwest Group, Beijing, China). The reaction time test is a classic test used to assess psychomotor speed. The subject was asked to press a button in his/her preferred hand, as quickly as possible, in response to the presentation of a stimulus. The stimulus was randomly selected from among two colors, i.e., red or green.

*Santa Ana dexterity test*. A plastic base plate with 12 pegs fitted in 4 rows was used. Each peg was removed, turned 180° and replaced in its slot. The objective was to turn as many pegs as possible in 30 s. The test was repeated twice for each hand. The number of pegs successfully turned was recorded as the test score. Mean scores were calculated separately for the preferred and non-preferred hand.

*Digit span*. In the digit forward sequence the tester read groups of 3–8 numbers and the participant was requested to repeat each sequence exactly as they heard them. The digit backwards sequence ran from two to eight digits and the participant was requested to repeat them in the exact reversed order. The score was the total number of correct individual sequences relayed.

*Digit symbol test*. A worksheet contained a list of numbers that were associated with certain simple symbols and a list of random digits from one to nine with blank squares below each digit. The participant was required to fill the blank squares with the symbols paired to their corresponding digits, and to do so as quickly as possible within 90 s. The number of correct matches was recorded for each participant.

*Benton visual retention test*. A design was shown to the participant for 10 s and then removed from view. The participant was immediately asked to draw the design from recall using a sheet of paper of the same size as the design. The number of complete correctly recalled pictures was calculated.

*Pursuit aiming test*. The participant's task was to pencil one dot inside each circle following a pattern given on a printed PA test sheet. This task was to be performed as quickly as possible within 60 s. Two scores were taken, one for the number of circles containing dots (correct pursuit aiming) and the other for the number of circles with external dots (error pursuit aiming).

Experienced occupational physicians and trained personnel carried out the neurobehavioral tests, which took about 50–60 min per person. The testing method was explained by the examiners, as recommended by the WHO–NCTB operational guide (WHO, 1986), until the participant appeared to completely understand the procedure. To avoid inter-rater bias, one examiner scored each test item. An occupational physician experienced with the neuropsychological tests reviewed all test scores before the data analysis.

### 2.4. Event-related auditory evoked potential (P300) test

Long-term occupational exposure to vanadium may lead to impairment in neurocognitive processes reflected in the event-related potentials generated in an auditory oddball task. We used the amplitude and latency of the P300 and the behavioral outcome of the oddball task to measure cognitive function.

Of the NCTB study population ($n = 714$), 97 exposed workers and 50 unexposed workers were selected for P300 assessment. All the participants were male and aged 30–45 years old. This selection was based on the following considerations. First, workers in this age range usually have the length of service sufficient for health effect observation of vanadium toxicity. Second, if the ages of subjects are more than 45 years old, the ages may become an influential factor that undermines the neurobehavioral outcomes. Finally, since most of the front-line workers are male, this study recruits only male workers.

The P300 was recorded and averaged using a Medtronic keypoint portable (MK-NMP) 4 channel signal averaging machine. An auditory oddball paradigm (standard tone: 100 dB, 1000 Hz, probability 80%; target tone: 100 dB, 2000 Hz, probability 20%) was applied to elicit auditory event-related potential (ERP), conforming to the IFCN (International Federation of Clinical Neurophysiologists) guidelines. (Heinze et al., 1999) Tones were presented in a random order through headphones at random intervals ranging from 1 to 2 s. ERP was recorded over the Cz positions, while placing combined reference electrodes on the mastoids and the ground electrode on the Fpz position, conforming to international 10–20 scalp electrode placement standards. (Klem et al., 1999) At the electrode positions, the skin was prepared with skin prepping gel to obtain impedances of less than 5 K$\Omega$. The low-pass filter was set at 1 Hz and the high-pass filter was set at 30 Hz. Responses were amplified and averaged 150 times. Binaural rarefaction clicks were applied at 60 dB above hearing level using earphones.

The participants covertly counted the target tones and the total count was noted down at the end of the session. They were asked to sit in a chair and keep their eyes closed under slightly dimmed lighting during testing. At the end of signal acquisition, ERP measurements were made and P300 was quantified by one of the authors who was blind to the participant's exposure status. The maximum negative or positive peak in the fixed latency windows was determined. The P300 amplitude was obtained by measuring the difference between the $N_2$ and the P300 peaks.

## 3. Statistics

A total of 37 incomplete samples incurring in the NCTB test process were removed for statistical analyses. Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 11.0). The $t$-test, $\chi^2$ test, and analysis of covariance were used for analyses. All outlying values were individually evaluated to determine the reason for the unusually poor performance. Tests with scores or results that were not thought to be physiologically plausible were eliminated from the analysis. All known confounders (gender, age, and culture) were measured and taken into account in the analysis.

## 4. Results

### 4.1. NCTB test outcomes

Table 1 summarizes the neurobehavioral test outcomes by vanadium-exposed and control groups. In the POMS test, the scores of the anger-hostility, depression-dejection, and fatigue-inertia were significantly higher in the exposed group than those in the control ($p < 0.05$). Further stratifying the study cohorts by working year, it became clear that the changes in anger-hostility, fatigue-inertia and vigor-activity were more significantly in workers with working experience of more than 10 years than those working less than 10 years ($p < 0.05$, Table 2). There were no difference of the scores for confusion-bewilderment and tension-anxiety between exposure group and control group ($p > 0.05$).

The scores of the forward digit span and backward digit span (reflecting auditory memory) were lower in the in the exposed group than in the control group ($p < 0.05$). The digit symbol (reflecting perception/motion speed) and Benton visual retention (reflecting visual memory) scores were also lower in the exposed group than in the control group ($p < 0.05$). The fastest simple reaction time, slowest simple reaction time, and mean simple reaction time (reflecting motion cooperation and manual operation speed) tended to be longer in the exposed workers than in control workers, but the differences were not significant ($p > 0.05$). The error dot score (reflecting motion cooperation and manual operation accuracy) was higher in the exposed group than in the controls. The Santa Ana dexterity scores (reflecting manual

**Table 1**
Neurobehavioral test results by study group.

| Test item | Control group (mean ± SD) $n = 251$ | $V$ group (mean ± SD) $n = 463$ | $p$ |
|---|---|---|---|
| **Mood states** | | | |
| Anger-hostility | 21.12 ± 6.25 | 22.37 ± 7.57 | 0.044[a] |
| Confusion-bewilderment | 13.84 ± 3.53 | 13.98 ± 4.25 | 0.831 |
| Depression-dejection | 25.46 ± 7.59 | 27.57 ± 10.11 | 0.01[a] |
| Fatigue-inertia | 13.25 ± 3.88 | 14.15 ± 4.29 | 0.01[a] |
| Tension-anxiety | 16.2 ± 4.18 | 16.6 ± 5.4 | 0.293 |
| Vigor-activity | 19.62 ± 5.66 | 17.48 ± 6.03 | 0.000[a] |
| **Simple reaction time** | | | |
| Fastest time | 277.04 ± 54.78 | 281.76 ± 45.98 | 0.679 |
| Slowest time | 627.43 ± 260.57 | 660.90 ± 452.09 | 0.718 |
| Mean time | 376.98 ± 90.86 | 380.36 ± 114.33 | 0.191 |
| Error dot score | 0.37 ± 0.64 | 0.58 ± 0.87 | 0.000[a] |
| **Digit span** | | | |
| Digit span forward | 12.32 ± 1.56 | 11.42 ± 1.93 | 0.000[a] |
| Digit span backward | 6.88 ± 2.37 | 6.05 ± 2.14 | 0.007[a] |
| **Santa Ana dexterity** | | | |
| Preferred hand | 15.84 ± 2.76 | 15.48 ± 2.64 | 0.708 |
| Non-preferred hand | 14.74 ± 2.37 | 14.66 ± 2.55 | 0.748 |
| **Digit symbol** | 56.99 ± 10.53 | 51.13 ± 13.61 | 0.001[a] |
| **Benton visual retention** | 7.41 ± 1.63 | 6.86 ± 1.66 | 0.025[a] |
| **Pursuit aiming** | | | |
| Total pursuit aiming | 107.18 ± 24.72 | 104.18 ± 29.91 | 0.997 |
| Correct pursuit aiming | 87.20 ± 25.58 | 78.64 ± 27.69 | 0.016[a] |
| Error pursuit aiming | 19.98 ± 24.02 | 25.54 ± 25.79 | 0.014[a] |

Covariates: gender, age, culture.
[a] Significant $p$-value.

**Table 2**
Emotional test results by study group and V group length of service (mean ± SD).

| Mood states | <10 years of service | | | ≥10 years of service | | |
|---|---|---|---|---|---|---|
| | Controls ($n$ = 32) | V group ($n$ = 53) | $p$ | Controls ($n$ = 219) | V group ($n$ = 410) | $p$ |
| Anger-hostility | 19.4 ± 6.2 | 21.5 ± 5.3 | 0.155 | 21.5 ± 6.1 | 23.1 ± 8.1 | 0.033[a] |
| Confusion-bewilderment | 12.9 ± 2.9 | 14.1 ± 3.5 | 0.151 | 14.2 ± 3.5 | 14.4 ± 4.2 | 0.968 |
| Depression-dejection | 23.7 ± 6.8 | 26.7 ± 7.8 | 0.044[a] | 25.9 ± 7.6 | 27.3 ± 11.0 | 0.041[a] |
| Fatigue-inertia | 12.9 ± 5.1 | 14.5 ± 3.7 | 0.130 | 13.4 ± 3.5 | 14.4 ± 4.4 | 0.043[a] |
| Tension-anxiety | 15.2 ± 4.1 | 16.8 ± 4.7 | 0.148 | 16.6 ± 4.1 | 17.1 ± 5.7 | 0.542 |
| Vigor-activity | 22.2 ± 6.2 | 22.3 ± 6.0 | 0.996 | 19.6 ± 5.3 | 17.3 ± 6.1 | 0.008[a] |

Covariates: gender, age, culture.
[a] Significant $p$-value.

**Table 3**
Neurobehavioral function test results by study group and V group length of service (mean ± SD).

| Neurobehavioral function | <10 years of service | | | ≥10 years of service | | |
|---|---|---|---|---|---|---|
| | Controls ($n$ = 32) | V-group ($n$ = 53) | $p$ | Controls ($n$ = 219) | V-group ($n$ = 410) | $p$ |
| **Simple reaction time** | | | | | | |
| Fastest time | 257.4 ± 49.2 | 256.6 ± 32.5 | 0.957 | 275.5 ± 53.7 | 278.2 ± 40.0 | 0.875 |
| Slowest time | 613.6 ± 228.8 | 521.8 ± 198.4 | 0.051 | 603.1 ± 233.4 | 619.2 ± 284.4 | 0.889 |
| Mean time | 352.7 ± 68.8 | 329.4 ± 47.0 | 0.080 | 370.0 ± 81.9 | 369.0 ± 70.3 | 0.357 |
| Error dot score | 0.5 ± 0.7 | 0.6 ± 0.8 | 0.296 | 0.4 ± 0.6 | 0.6 ± 0.8 | 0.005[a] |
| **Digit span** | | | | | | |
| Digit span forward | 12.6 ± 1.5 | 11.2 ± 1.5 | 0.018[a] | 12.4 ± 1.5 | 11.5 ± 1.8 | 0.000[a] |
| Digit span backward | 7.5 ± 2.5 | 7.2 ± 2.4 | 0.564 | 7.0 ± 2.4 | 6.3 ± 2.2 | 0.033[a] |
| **Santa Ana dexterity** | | | | | | |
| Preferred hand | 15.7 ± 2.3 | 16.4 ± 2.6 | 0.190 | 16.1 ± 2.8 | 15.8 ± 2.6 | 0.623 |
| Non-preferred hand | 15.2 ± 2.3 | 15.1 ± 2.3 | 0.966 | 14.8 ± 2.4 | 14.8 ± 2.5 | 0.624 |
| **Digit symbol** | 64.5 ± 11.9 | 65.9 ± 10.4 | 0.337 | 57.9 ± 8.8 | 53.5 ± 11.7 | 0.001[a] |
| **Benton visual retention** | 7.7 ± 1.4 | 7.8 ± 1.3 | 0.695 | 7.5 ± 1.6 | 7.0 ± 1.6 | j |
| **Pursuit aiming** | | | | | | |
| Total pursuit aiming | 117.1 ± 26.1 | 114 ± 35.9 | 0.586 | 108.1 ± 24.2 | 108.1 ± 28.4 | 0.570 |
| Correct pursuit aiming | 84.8 ± 32.8 | 91.4 ± 28.0 | 0.299 | 90.1 ± 24.3 | 84.1 ± 25.7 | 0.080 |
| Error pursuit aiming | 32.3 ± 34.5 | 28.6 ± 25.1 | 0.061 | 18.0 ± 22.0 | 24.1 ± 24.6 | 0.013[a] |

Covariates: gender, age, culture.
[a] Significant $p$-value.

operation agility) were not obviously different between the two groups ($p > 0.05$). The correct pursuit aiming (reflecting motion stability and speed) scores were lower in the exposed group than in the control group (Table 2). A service year-related decline in neurobehavioral functions was also observed in several functional tests. For example, the scores of the digit span backward test and Benton visual test were not significantly changed in workers whose service years were less than 10 years but they became significantly altered among workers who served more than 10 years in the job (Table 3).

**Table 4**
P300 performance by study group.

| Items | Controls (mean ± SD), ($n$ = 50) | V group (mean ± SD), ($n$ = 97) | $p$ |
|---|---|---|---|
| Latency (ms) | 295.01 ± 30.43 | 306.69 ± 33.24 | 0.035[a] |
| Amplitude (μV) | 3.71 ± 1.83 | 3.48 ± 2.69 | 0.589 |
| Slowest button reaction time (ms) | 590.63 ± 121.49 | 623.88 ± 161.95 | 0.165 |
| Fastest button reaction time (ms) | 272.65 ± 63.91 | 277.17 ± 59.55 | 0.671 |
| Mean button reaction time (ms) | 391.24 ± 80.33 | 409.14 ± 87.29 | 0.192 |
| Counting errors[a] | 0.87 ± 1.82 | 1.49 ± 2.51 | 0.008[a] |

[a] Counting errors: the difference between the tone count made by the subject and the actual target count; significant $p$-value.

### 4.2. P300 test outcomes

The P300 parameters among the exposed group and the control group are shown in Table 4. The P300 latencies of the exposed workers were significantly longer as compared to those of the controls ($p = 0.035$). The P300 amplitudes of the exposed workers tended to be lower than those of the controls, but the difference did not reach statistical significance ($p = 0.589$). Similarly, the mean button reaction time, fastest button reaction time and slowest button reaction time tended to be longer in the exposed group than in the control group, but the differences were not significant ($p > 0.05$). The exposed workers made significantly more counting errors than did the control workers ($p = 0.008$) in the auditory oddball task.

## 5. Discussion

Behavioral toxicology has become increasingly important in the risk assessment of exposure to neurotoxic substances because the high sensitivity of behavior toward neurotoxic action allows for a more sensitive measure of neurotoxic outcomes in exposed populations (Yuan et al., 2006). The results presented in this human study clearly indicate that occupational exposure to vanadium led to the measurable changes in workers' neurobehavioral performance.

Vanadium could induce oxidative stress in the central nervous system, and the main areas affected by vanadium-mediated freeradical generation were the hippocampus and the cerebellum. Also, the morphological alterations of neurons and astroglial cells in adult rat central nervous system after vanadium exposure was reported (Garcia et al., 2005). Vanadium can exert neurotoxic effects in dopaminergic neuronal cells via caspase-3-dependent PKCδ cleavage (Afeseh Ngwa et al., 2009). Vanadium exposure through lactation would induce neurotoxicity in rat developing CNS (Soazo and Garcia, 2007). Vanadium inhalation produces a time dependent loss of dendritic spines, necrotic-like cell death, and alterations of the hippocampus neuropile, which correlate with spatial memory impairment (Avila-Costa et al., 2006). Noticeably, the concentrations used in these animal studies are higher than the concentrations of human occupational exposure, although animal models make it possible to observe the impact of vanadium on the nervous system function. The current study, by using NCTB and P300 measurements, extends our animal research and allows us to verify the neurotoxic effects of vanadium in humans with occupational exposure. This study made full use of our geographical advantage and was carried out in one of the largest vanadium-containing production manufacturers in China. The factory's more than 20 years of production experience have laid a good foundation for this study.

Our data indicated that vanadium exposure was associated with increased negative emotion and decreased positive emotion in exposed workers. The negative emotions in the exposed workers, such as anger-hostility, depression-dejection and fatigue-inertia, were stronger than those of the controls. Vanadium exposure was also associated with decreased coordination, short-term memory and reaction speed. Workers exposed to vanadium performed worse in tests of auditory memory, perception/motion speed, motion cooperation and manual operation speed and accuracy than the control group. The simple reaction time of the vanadium-exposed workers was longer than that of the controls, which is consistent with the mean button reaction time tested with the P300. Overall, these differences in the emotion and behavior of vanadium-exposed workers compared with non-exposed workers indicate an association with occupational exposure to vanadium.

The question as to how exactly vanadium causes these neurobehavioral alterations remain elusive. It is known that disorders that affect the primary cognitive operations of attention allocation and immediate memory are associated with P300 measures with a reduced amplitude and/or increasing latency (Polich and Herbst, 2000; Johnson, 1995). At the cellular level, a recent study by Kanthasamy group suggests that vanadium may exert neurotoxic effects in dopaminergic neuronal cells via caspase-3-dependent PKCdelta cleavage. The authors provide the evidence that vanadium is taken up by DA neurons via a DMT1 mediated transport system. Within the cells, vanadium activates caspases pathway, possibly promoting the nigral dopaminergic degeneration (Afeseh Ngwa et al., 2009). Other investigators have suggested that vanadium may act as phosphate analog to interfere with ATPase, phosphatases, and phosphate-transfer enzymes, or as a neuroendocrine disruptor. However, the current literature provides little information regarding the mechanism of vanadium toxicity in vivo (USCDC, 2009). Since vanadium is widely used in industrial applications, the increased concentration of this element in the atmosphere due to rapid development of industry demands a thorough evaluation of its toxicity and related mechanism of action. Our present data suggest a clear neurotoxic effect of vanadium at low exposure levels in humans. Thus the potential of vanadium to induce chronic neurological disorders following exposure should be further investigated. In addition, the limits for occupational exposure to vanadium in the workplace should be established.

A limitation of our study was that in order to ensure the test quality, each study participant was required to take the P300 test for three times, which was time-consuming and led to a low compliance and a limited sample size. As a result, male workers aged 30–45 years were chosen for our P300 test, because of their relative seniority and good participation rate and because of the suitable age range to test human behavior with the minimal age effect (Volkow and Gur, 1998; Mozley et al., 1999). Therefore, choosing workers between 30 and 45 years of age in this study, while ensured a sufficient duration of occupational exposure and the participation rate, may miss the subtle changes among workers outside of this age range. Since this study did not measure air levels of lead and manganese, our observation may be limited by the interference due to co-exposure to these two metals commonly seen in the workplace.

In summary, our findings suggest that occupational exposure to vanadium appears to result in altered neurobehavioral functions. Vanadium exposure among exposed steel workers affects the normal neuronal functions including emotion, cognition, attention, short-term memory, reaction speed, accuracy and coordination. Some of these changes are also associated with workers' years of service. This study provides the initial epidemiological evidence demonstrating the association between vanadium exposure and altered neurobehavioral function. The findings are of value for policy makers to prevent vanadium exposure in workplace. The data may be of value for environmental exposure assessment of vanadium neurotoxicity.

## Conflict of interest

The authors report no conflicts of interest.

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