*Toxicology and Industrial Health* 2001; **17**: 128–137

www.tihjournal.com

# Cognitive performance and cerebrohemodynamics associated with the Persian Gulf Syndrome

LEONID BUNEGIN,[a] HOWARD C MITZEL,[a] CLAUDIA S MILLER,[b] JERRY F GELINEAU[a] AND GLEB P TOLSTYKH[a]

[a]*Department of Anesthesiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229, USA*
[b]*Department of Family and Community Medicine, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229, USA*

The Persian Gulf Syndrome generally manifests as a set of nonspecific complaints with emphasis on central nervous system impairment. The purpose of this study was to determine if cognitive performance and middle cerebral artery blood flow velocity (MCABFV) were altered in symptomatic Gulf War veterans (sGWVs) and asymptomatic Gulf War veterans (aGWVs) by exposure to low levels of acetone. MCABFV was assessed in male aGWVs ($n$=8) and sGWVs ($n$=8) during cognitive challenges while breathing 1) clean air, 2) a clean air placebo, and 3) a mixture of air and 40 parts per million (ppm) acetone. Pulmonary function was also evaluated. Pulmonary function tests showed no statistical differences between aGWVs and sGWVs while breathing clean air or 40 ppm acetone in air. Cognitive performance was similar during the clean air, placebo, and acetone test conditions for sGWVs and aGWVs. Data pooled across test conditions for each group indicated a statistically significant ($P<0.05$) poorer performance primarily in memory and executive function tasks by sGWVs. sGWVs had a 34.2% higher baseline MCABFV than aGWVs ($P<0.05$). Increases in MCABFV for aGWVs (averaged over all cognitive tasks for each test condition) ranged between 7.8% and 8.8%, and were not statistically significant. Increases for sGWVs ranged between 0.3% and 4.8%, averaged over all cognitive tasks for each test condition. No significant differences were noted between the clean air and placebo test conditions but both were significantly different compared to the acetone condition. Differences in MCABFV increases for each of the test conditions between aGWVs and sGWVs were also statistically significant. sGWV did not appear to demonstrate pulmonary dysfunction following exposure to acetone. They did, however, appear to have generally lower cognitive function as compared to aGWVs. sGWVs appeared to have a significant degree of autoregulatory disruption in cerebral perfusion, resulting in reduced cognitive reserve capacity and potentially impaired ability to handle complex cognitive tasks. *Toxicology and Industrial Health* 2001: **17,** 128–137.

**Key words:** *blood flow; brain; cognition; Doppler; Gulf; Persian; trancranial; war*

## Introduction

Thousands of servicemen and women returned from the Persian Gulf with chronic, polysymptomatic, multisystem conditions of indeterminate origins and etiologies. Common symptoms include fatigue, sleep disturbance, forgetfulness, joint pain, irritability, difficulty in concentrating, depression, headache, rash, cough, abdominal pain, and diarrhea (Anonymous, 1997; Escalante and Fischbach, 1998; Fukuda et al., 1998; Kroenke et al., 1998; Roy et al., 1998; Gray et al., 1999; Kaires, 1999; McCauley et al., 1999; Miller and Ashford, 1999). Surveys of symptom patterns in these veterans suggest many nonspecific complaints with notable emphasis on central nervous system dysfunction (Goldstein et al., 1996; Axelrod and Milner, 1997; Haley et al., 1997a; 1997b; Sillanpaa et al., 1997; Anger et al., 1999; Engel et al., 1999; Ishoy et al., 1999; Suadicani et al., 1999). In clinical interviews, some veterans of the Gulf War report acquired intolerance for alcohol, drugs, and chemical odors such as nail polish remover and vehicle emissions (Bell et al., 1990; Miller and Prihoda, 1999). These chemical odors are reported to trigger neurobehavioral symptoms such as confusion, difficulty concentrating, memory loss, and headache (Bell et al., 1990; Miller and Prihoda, 1999). A striking feature of these reports is that these symptoms appear to abate following withdrawal from the putative source odor (Bell et al., 1990; Miller and Prihoda, 1999). The apparent CNS involvement and transitory nature of the reported symptoms are reminiscent of symptoms associated

1. Abbreviations: MCABFV, middle cerebral artery blood flow velocity; CBF, cerebral blood flow; CVR, cerebrovascular resistance; sGWVs, symptomatic Gulf War veterans; aGWVs, asymptomatic Gulf War veterans; FEV, forced expiratory volume; VC, vital capacity; MMFR, maximum mid-expiratory flow rate; TCD, transcranial Doppler; $V$, minute ventilation; RR, respiratory rate; He, Hand–Eye Coordination; Rt, Simple Reaction Time; Df, Visual Digit Span forward; Db, Visual Digit Span backward; Ha, Horizontal Addition; Pm, Pattern Memory; Ss, Switching Attention, side to side; Sd, Switching Attention, side direction; Sds, Switching Attention, side direction side

2. Address all correspondence to: Leonid Bunegin, BS, Department of Anesthesiology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229, USA
E-mail: bunegin@uthscsa.edu



10.1191/0748233701th100oa

with transient ischemia. During transient ischemia, blood flow to areas of the brain is temporarily reduced resulting in mental confusion, memory loss, and blurred vision (Jensen and de Fine Olivarius, 1981; Santoloci et al., 1985; Kritchevsky, 1987; Yamane et al., 1989; Weinachter et al., 1990).

Accordingly, this study was designed as a controlled experiment to determine if cognitive performance and middle cerebral artery blood flow velocity (MCABFV) were altered in symptomatic Gulf War veterans (sGWVs) and asymptomatic Gulf War veterans (aGWVs) by exposure to low levels of acetone.

Physiological parameters such as MCABFV and pulmonary function were monitored while simultaneously collecting indicators of cognitive function in reaction trials and performance in short-term memory tasks. These tasks were chosen to activate cerebral metabolic activity so as to stimulate cerebral blood flow (CBF) as well as to reflect the reported symptoms of confusion, difficulty in thinking, and memory loss.

## Methods

*Subjects*

Following Institutional Review Board approval, subjects were recruited with newspaper advertisements and through the Audie L. Murphy Memorial Veterans Hospital at San Antonio, TX. All respondents were first telephone-screened for the following inclusion criteria: 1) nonsmoking, nonactive military males aged 18–55 years with service (Army) in the Persian Gulf theater between 1989 and 1991; and 2) absence of any major medical conditions including asthma, high blood pressure, or heart problems and not taking any medications. In addition, all subjects were asked for 3) an open-ended description of symptoms, followed by a specific checklist for headache, diarrhea, difficulty concentrating, memory loss, or aching joints; and 4) an open-ended description of acquired illness to chemical odors followed by a specific checklist that included engine exhaust and nail polish remover. To be admitted to the study, sGWVs had to report at least one neurobehavioral symptom, such as, difficulty concentrating, memory loss, confusion, as well as, an acquired negative reaction to chemical odors. Asymptomatic controls were admitted if they identified themselves as such early in the screening process and reported the absence of any symptoms associated with Gulf War Illness and the absence of negative responses to everyday chemical odors.

Only males were admitted to the study. First, the selected test battery was normed on male industrial workers. Many neuropsychological tests have differing norms for males and females, which would indicate

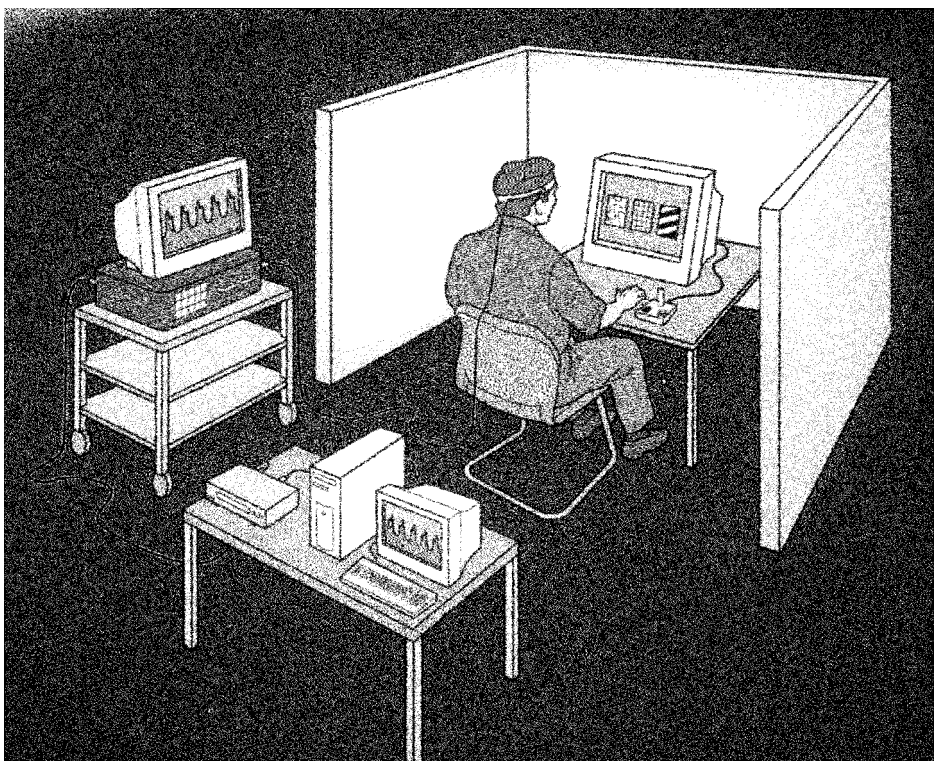

**Figure 1.** Experimental set-up depicting the testing area and location of the monitoring equipment.

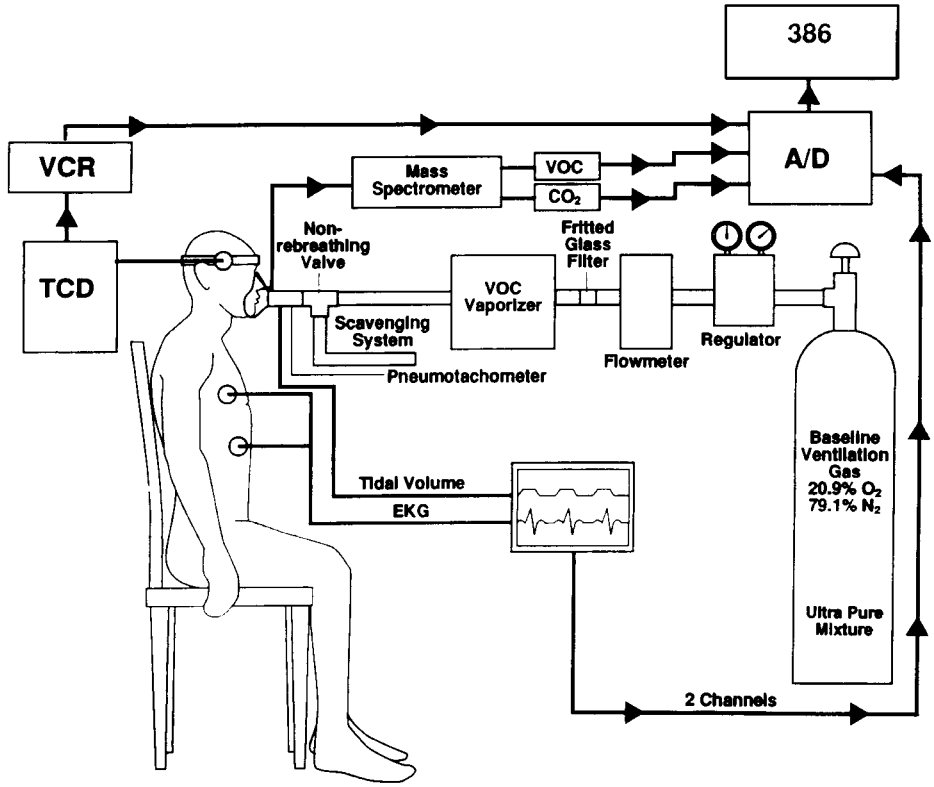


**Figure 2.** Schematic representation of subject instrumentation, gas delivery system, monitoring, and data recording systems.

that a separate sampling partition would be required for female participants. Secondly, males represented an overwhelming majority of Gulf War veterans, and the available pool of symptomatic female subjects was uncertain. Following informed consent, subjects were offered US$25.00 to complete the study.

*Design and protocol*

Two independent groups of eight subjects each were evaluated under three test conditions *via* microcomputer. Each test condition consisted of multiple trials on nine cognitive tasks. The first test condition was a baseline state with the subjects breathing clean air. The second was a placebo, where the subjects were told they would be breathing a subodor threshold of acetone in air when in reality they were breathing clean air. During the third test condition, subjects were exposed to 40 parts per million (ppm) acetone in air. The ordering of the cognitive tasks and the number of trials in each task were identical for each of the three test conditions. A 2-min rest period was provided between test conditions 1 and 2. Symptom reports were collected between conditions 2 and 3 while acetone exposure was initiated so as to provide a 3- to 4-min equilibration period. MCABFV, end-tidal $CO_2$, and acetone concentrations in inspired and expired air were monitored continuously. A pulmonary function battery measuring forced expiratory volume (FEV), vital capacity (VC), and maximum mid-expiratory flow rate (MMFR) (West, 1975) was administered prior to the first test condition and immediately following the third test condition.

A 2-MHz transcranial Doppler (TCD) probe was secured over the temporal window of the nondominant side by a headband. The probe was adjusted so that a maximal MCABFV profile was obtained. Subjects were then seated at a standard PC keyboard and joystick facing a flat screen monitor (Model NEC 3FGX) adjusted to eye level. The keyboard was covered, exposing only the number pad at the right. The joystick was positioned at the

**Table 1.** Subject profiles; self-assessments.

| Branch | aGWVs army | sGWVs army |
|---|---|---|
| Age (Years) | 30.1 ± 6.7 | 36.8 ± 9.6 |
| Education (Years) | 13.50 ± 1.69 | 14.13 ± 3.09 |
| Handedness | 1.25 ± 0.46 | 1.25 ± 0.46 |
| Tension | 1.33 ± 0.26 | 2.11 ± 0.48* |
| Depression | 1.38 ± 0.35 | 2.94 ± 0.68* |
| Anger | 1.37 ± 0.36 | 2.42 ± 0.84* |
| Fatigue | 1.69 ± 0.37 | 3.06 ± 0.78* |
| Confusion | 1.56 ± 0.56 | 2.31 ± 0.65* |

Data represented as mean ± SD. Self asessment scale: 0–5, 0–least affected, 5–most affected. Handedness: 1=right, 2=left.
*Indicates $P < 0.05$.

**Table 2.** Pulmonary function tests administered to aGWVs and sGWVs during exposure to clean air and 40 ppm acetone.

| | Clean air | | Acetone | |
|---|---|---|---|---|
| | aGWV | sGWV | aGWV | sGWV |
| *V*, l/min | 6.8±2.4 | 5.2±1.8 | 7.2±2.9 | 5.7±2.4 |
| RR, breaths/min | 16.1±1.6 | 16.5±4.3 | 15.4±2.1 | 15.9±4.6 |
| $ETpCO_2$, mm Hg | 37±4 | 39±8 | 41±7 | 39±8 |
| MMFR, l/s | 2.51±0.68 | 2.71±1.37 | 3.58±1.01 | 2.4±2.4 |
| FEV/VC, % | 61±18.1 | 62.2±12.4 | 47.8±17.8 | 69±12.9 |
| BP, mm Hg | 96.5±5.6 | 97.2±3.3 | 101.5±6.3 | 96±7.8 |
| Acetone, ppm | 0 | 0 | 49±21 | 46±22 |

Data represented as mean±SD.

preferred hand. The wall that subjects faced was draped by brown paper, which encased the monitor such that only the screen was showing. Subjects' peripheral vision was obscured by two 6-ft-high gray panels on either side, forming a bay open at the back and above (Figure 1). A silicone face mask covering the nose and mouth was positioned on the subjects. A stainless steel spirometer incorporated within the mask measured the pulmonary battery and ventilation parameters, minute ventilation (*V*), and respiratory rate (RR). Additionally, a small silicone sampling tube located at the nostril level conveyed inspired and expired breathing gas to a quadrupole mass spectrometer for end-tidal $CO_2$ and acetone analysis. Ultrapure breathing gas was conveyed to the subject through a silicone ventilation circuit incorporating a regulator, flowmeter, and a solvent vaporizer. Expired gas exited through a nonrebreathing valve and was vacuum-scavenged (Figure 2). Elements of the delivery system were made either of stainless steel or brass so as to eliminate potential offgassing contaminants. The face mask and ventilation circuit were washed with nonscented biodegradable soap, rinsed with deionized water, and baked for 24 h at 150°C so as to insure that all volatiles were driven off. A Medasonics TRANSSPECT® was used to continuously monitor MCABFV. Output was recorded to standard videotape indexing the start and end of each cognitive task. Data were digitally acquired and analyzed off line at a later time. Mean flow velocities were continuously sampled at 1-s intervals during the cognitive testing. Prior to and following each test condition, MCABFV was recorded for 1 min while the subject sat quietly with his eyes shut in order to establish and verify baselines. Data from the mass spectrometer/spirometer were also digitally acquired using LabWindows®. The computer CPU, exposure apparatus, mass spectrometer, TCD, and recording equipment were positioned outside the panels and could not be seen by the subjects. Access to the testing area was barred, and strict quiet was maintained.

A brief physical examination was also performed before testing, measuring blood pressure, heart rate, as well as evaluating the heart and breathing by auscultation. Subjects were also ascertained to be medication- and caffeine-free for at least 24 h. Prior to starting the tests, all subjects performed a practice session with cognitive tasks to familiarize them with the instructions and to minimize practice effects. Following the experiment, an in-depth interview was conducted with each participant detailing medical history and experiences in terms of potential chemical exposures during the Gulf War.

*Cognitive measurements*

The NES2® battery of neuropsychological tests (Letz, 1991) was chosen for this study based on the following criteria: 1) computer-based administration and scoring; 2) validity for assessing cognitive impairment, including normative data; 3) previous use in toxicological and chemical exposure studies; 4) parallel forms for repeat testing; 5) measurement of primary domains of interest, including attention, memory, and executive function; 6) inability of subjects to make verbal responses during test administration due to presence of the face mask used for gas delivery. All tests and instructions were computer-administered, timed, and scored, thereby increasing reliability and sensitivity to subtle cognitive changes. In order of presentation, the following tasks were presented: Hand–Eye Coordination (He), Simple Reaction Time (Rt), Visual Digit Span Forward (Df) and Visual Digit Span Backward (Db), Horizontal Addition (Ha), Pattern Memory (Pm), Switching Attention, Side to Side (Ss); Switching Attention, Side Direction (Sd); Switching Attention, Side Direction Side (Sds). These tasks represented memory (Df, Db, Pm), cognitive (Ha, Ss, Sd, Sds), and perceptual–motor (He, Rt) challenges. The

**Table 3.** Mean arterial pressure (mm Hg) for aGWVs and sGWV prior to the start and at the end of cognitive testing.

| | Before cognitive testing | After cognitive testing |
|---|---|---|
| aGWVs | 97.2±3.3 | 96.0±7.8 |
| sGWVs | 96.5±5.6 | 101.5±6.3 |

No significant changes in mean pressure were noted. Data represented as mean±SD.

**Table 4.** Mean time in minutes needed for completion of all cognitive tasks for each test condition.

| | Clean air | Sham | Acetone |
|---|---|---|---|
| aGWVs | 14.2±1.9 | 14.2±2.4 | 14.5±2.0 |
| sGWVs | 15.0±3.5 | 14.6±2.7 | 14.6±3.0 |

The acetone block also represents mean duration of acetone exposure. Data represented as mean±SD.

number of correct responses and/or response latency were measured for each test.

Data are expressed as means and standard deviations, and statistical significance was assessed by two-way analysis of variance with repeated measures where appropriate, followed by Student–Neuman–Kuels posttests, where indicated. Criterion for rejecting the null hypothesis was set at $P<0.05$.

## Results

The study was conducted in 1994, approximately three years following the Persian Gulf War and was completed in less than four months. Ages of the respondents ranged between 22 and 40 years for aGWVs and 25 and 48 years for sGWVs. There were no significant differences in either the educational level, handedness, age, or branch of service between aGWVs and sGWVs. However, sGWVs self-assessed as experiencing more tension, depression, anger, fatigue, and confusion (Table 1). A large percentage of sGWVs (62.5%) reported sensitivity to acetone-based finger nail polish remover, whereas none of the aGWVs reported this sensitivity. Pulmonary function tests showed no statistical differences between aGWVs and sGWVs while breathing clean air or 40 ppm acetone in air (Table 2). There were also no significant changes in mean arterial pressure between the start and end of the test periods for either group, nor were there significant differences between groups (Table 3).

The total time needed for completion of the cognitive tasks for each test condition is summarized in Table 4. No significant differences were detected between test conditions within subject groups or across subject groups. Mean acetone exposure time was essentially identical for aGWVs (14.5±1.9 min) and sGWVs (14.6±3.0 min).

No significant differences were noted in the number of correct responses for the individual cognitive tasks among the clean air, placebo, or acetone test conditions for sGWVs or aGWVs (Table 5). However, pooled data across the three test conditions indicated a statistically lower number of correct responses primarily in memory (Df, Db, Pm) and executive function (Sds) tasks by sGWVs (Table 5).

No significant differences were also noted in performance latency for the Rt, Pm, Ss, and Sd tasks when comparing the clean air, placebo, and acetone test conditions for sGWVs or aGWVs. Ha and Sds latencies were, however, significantly greater for sGWVs compared to aGWVs. Pooled data across the three test conditions suggested significantly longer latencies for Ha, Ss, Sd, and Sds in sGWVs than in aGWVs (Table 6).

Baseline MCABFVs at the start and end of each test condition were not significantly different for either aGWVs or sGWVs. Overall, sGWVs had a statistically higher (on average 34.2%) baseline CBFV than aGWVs (Table 7). Increases in MCABFV for aGWVs (averaged over all cognitive tasks for each test condition) ranged between 7.8% and 8.8%, and were not statistically significant.

**Table 5.** Cognitive performance scores in aGWVs and sGWVs.

| | aGWV | | | sGWV | | |
|---|---|---|---|---|---|---|
| | Clean | Sham | Acetone | Clean | Sham | Acetone |
| He | 2.12±0.46 | 2.02±0.52 | 1.85±0.48 | 2.01±0.42 | 1.97±0.35 | 2.08±0.54 |
| Pooled | | **2.12±0.76** | | | **2.02±0.41** | |
| Df | 7.00±1.07 | 7.63±1.06 | 7.38±1.19 | 6.38±1.69 | 6.38±1.41 | 6.13±1.96 |
| Pooled | | **7.33±1.09*** | | | **6.29±1.63*** | |
| Db | 5.63±1.69 | 6.13±0.99 | 6.50±1.20 | 4.88±0.83 | 5.13±1.89 | 4.88±1.81 |
| Pooled | | **6.38±1.32*** | | | **4.95±1.52*** | |
| Pm | 13.38±1.19 | 12.38±1.69 | 12.63±1.30 | 12.13±1.13 | 10.63±3.46 | 10.88±2.03 |
| Pooled | | **12.95±1.39*** | | | **11.14±2.50*** | |
| Ha | 18.50±1.60 | 19.25±1.04 | 18.25±1.82 | 18.38±2.20 | 18.50±0.93 | 18.13±2.23 |
| Pooled | | **18.67±1.34** | | | **18.33±1.81** | |
| Ss | 19.63±0.74 | 20.00±0.00 | 19.88±0.35 | 19.50±0.76 | 19.88±0.35 | 20.00±0.00 |
| Pooled | | **19.83±0.37** | | | **18.96±1.22** | |
| Sd | 18.87±1.04 | 19.63±0.52 | 19.38±0.74 | 19.00±0.93 | 19.13±1.36 | 18.75±1.39 |
| Pooled | | **19.29±0.77** | | | **19.79±0.36** | |
| Sds | 29.63±0.92 | 29.75±1.39 | 30.25±1.58 | 26.63±4.27 | 27.75±4.13 | 28.38±4.17 |
| Pooled | | **29.88±1.30*** | | | **27.58±4.19*** | |

No statistically significant differences in performance scores between aGWVs and sGWVs were noted for corresponding test conditions. Data represented as mean±SD.

*Indicates $P<0.05$ for data pooled across the test blocks: aGWVs versus sGWVs.

**Table 6.** Cognitive performance latencies in milliseconds for aGWVs and sGWVs.

| | aGWV | | | sGWV | | |
|---|---|---|---|---|---|---|
| | Clean | Sham | Acetone | Clean | Sham | Acetone |
| RT | 276.00±42.76 | 271.50±38.45 | 263.75±40.95 | 270.38±36.12 | 300.13±52.23 | 297.88±59.89 |
| Pooled | | **270.29±39.44** | | | **289.46±50.10** | |
| PM | 4.62±0.93 | 4.24±0.49 | 4.21±0.73 | 4.77±3.04 | 4.46±1.64 | 4.19±1.55 |
| Pooled | | **4.36±0.22** | | | **4.47±2.08** | |
| HA | 2.57±0.54* | 2.57±0.62* | 2.56±0.63* | 3.71±0.97* | 3.86±0.76* | 4.25±1.36* |
| Pooled | | **2.56±0.57**† | | | **3.94±1.04**† | |
| SS | 285.50±31.41 | 287.75±33.76 | 295.63±30.78 | 327.50±60.63 | 367.88±115.54 | 344.63±94.21 |
| Pooled | | **285.46±27.09**† | | | **346.67±90.38**† | |
| SD | 388.63±58.54 | 414.88±48.00 | 402.75±50.44 | 496.38±103.91 | 96.38±103.91 | 482.88±63.21 |
| Pooled | | **402.08±51.37**† | | | **473.38±116.67**† | |
| SDS | 520.63±57.77* | 492.75±48.38* | 484.50±47.66* | 744.38±192.17* | 631.88±137.76* | 632.75±105.65* |
| Pooled | | **488.25±37.12**† | | | **716.60±160.45**† | |

Data represented as mean±SD.
*Indicates significant difference at $P<0.05$: aGWVs versus corresponding sGWVs test condition.
†Indicates $P<0.05$ for data pooled across test blocks: aGWVs versus sGWVs.

Increases for sGWVs ranged between 0.3% and 4.8% averaged over all cognitive tests for each test condition. No significant differences were noted between the clean air and placebo test conditions, but both were significantly different compared to the acetone condition. Differences in MCABFV increases for each of the test conditions between aGWVs and sGWVs were also statistically significant (Figure 3).

## Discussion

The concept that Gulf War Syndrome is purely a psychological condition without some level of physiologic basis is clearly unsupported by a mounting accumulation of reports in the current literature (Goldstein et al., 1996; Anonymous, 1997; Axelrod and Milner, 1997; Haley and Kurt, 1997; Haley et al., 1997a; 1997b; Fukuda et al., 1998; Anger et al., 1999; Engel et al., 1999; Ishoy et al., 1999; Suadicani et al., 1999). While self-assessment surveys provide little in terms of etiology, they are valuable in establishing the spectrum of the illness, allowing specific hypotheses to be generated for detailed testing.

**Table 7.** Baseline CBFV at the start and end of each cognitive testing block for aGWVs and sGWVs.

| | aGWVs | | sGWVs | |
|---|---|---|---|---|
| | Start | End | Start | End |
| Clean air (cm/s) | 31.49±1.18* | 33.54±0.65† | 44.36±0.80* | 44.32±1.81† |
| Sham (cm/s) | 33.54±0.65* | 31.79±0.76† | 43.32±1.81* | 44.23±1.03† |
| Acetone (cm/s) | 32.88±0.52* | 31.73±0.62† | 43.95±0.81* | 42.35±0.71† |
| Pooled (cm/s) | 32.50±0.9* | | 43.60±0.7* | |

Data represented as mean±SD.
*Indicates a significant difference at $P<0.05$ between starting CBFVs for aGWVs and sGWVs.
†Indicates a significant difference at $P<0.05$ between ending CBFVs for aGWVs and sGWVs.

Of the 33 respondents who indicated a willingness to participate in this study, eight aGWVs and eight sGWVs fulfilled the entrance criteria and completed all tests. The sample size model of Norman and Streiner (1994) was applied in order to determine the smallest change in cognitive function that could be detected with the available subject population. Model parameters called for a 95% confidence interval at a 90% power level. The average measurement variability in cognitive task scores in this study was 17% and was consistent with the variability of the cognitive task scores estimated from a literature survey of organic solvent exposure studies that used the NES2® battery to assess cognitive function in adults (Hudnell et al., 1996; White et al., 1996; Mulder et al., 1999; Sethre et al., 2000). Applying a 17% measurement variability to the Norman and Streiner sample size model indicated that the smallest reliable change in cognitive function that could be detected in this study would be 19%. Applying the same analysis to MCABFV measurements at an identical confidence interval and power as the cognitive measurements, and a TCD measurement variability of 3.1%, the smallest reliable change in MCABFV that could be detected in this study would be 3.5%.

In interviews with sGWVs, Miller and Ashford (1999) found that almost 90% of the respondents reported that exposure to organic solvents such as gasoline, exhaust, and notably 'nail polish remover' in the course of normal daily activity triggered symptoms of confusion, memory loss, and difficulty in thinking. Since the primary component of many finger nail polish removers is acetone, the exposure level in this study was set so that it was low enough to not overly trigger symptoms in sGWVs, yet high enough to produce measurable effects, should they exist, in physiological and cognitive performances.

While odors have been shown to stimulate increases in CBF, the concentration of odorant needed to elicit the response was on the order of 30 times the olfactory

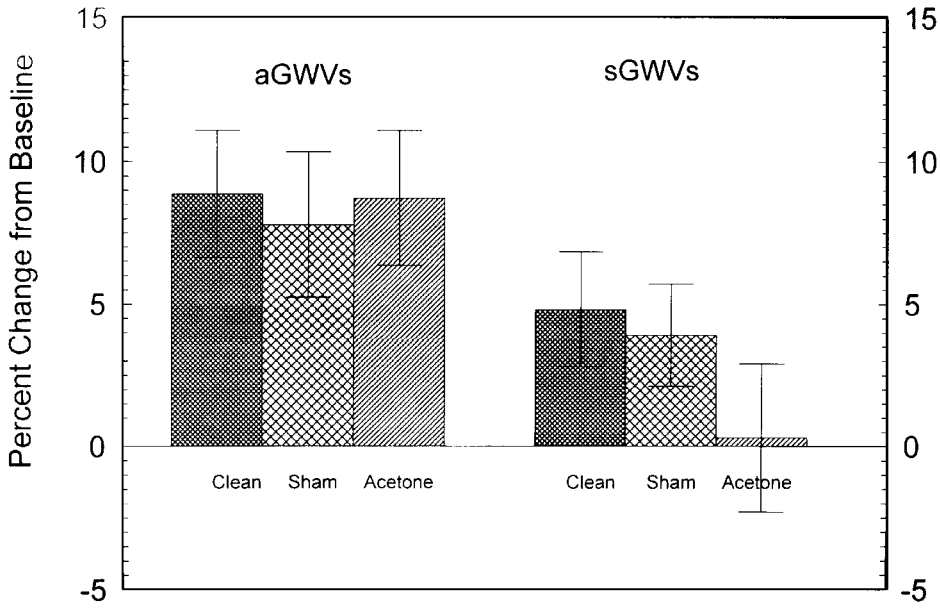


**Figure 3.** CBF velocity changes in Gulf War veterans during cognitive challenge. The difference in increase in MCBFV in response to cognitive challenge for corresponding test conditions for aGWVs versus sGWVs was statistically significant at $P > 0.05$. In sGWVs, exposure to acetone significantly depressed MCABFV response ($P > 0.05$).

detection limit (Zattore et al., 2000). The exposure level of 40 ppm used in this study was only twice the lower limit of olfactory detection, and 250 times lower than the lower limit of irritancy (Arts et al., 2002). This exposure level was detected by all subjects, yet appeared to avoid pharmacologic and cerebrohemodynamic effects. Basal MCABFV did not change in response to acetone exposure in this study for either subject group.

The acetone exposures in this study had no effect on pulmonary function in the aGWVs. Surprisingly, however, no effects were also observed in the sGWVs – a finding that differs from several anecdotal reports of shortness of breath, increased RR, and difficulty breathing (Anonymous, 1997; Joseph, 1997; Gray et al., 1999; Miller and Ashford, 1999). Exit interviews with the subjects indicated that 50% of the sGWVs felt 'warmth to burning' and/or 'congestion in the lungs' – symptoms that could not be confirmed by auscultation or by pulmonary function tests. Conversely, none of the aGWVs reported pulmonary complications or symptoms. Additionally, 37.5% of the sGWVs indicated that performing the cognitive test battery under acetone exposure was significantly more difficult than when breathing clean air, while aGWVs felt that performing the cognitive tests during acetone exposure was not any more difficult than during clean air test conditions. The aGWVs performed equally well while breathing acetone as during the clean air and placebo test conditions. The sGWVs also performed equally well under the influence of acetone and while breathing clean air. These observations allude to the possibility that sGWVs may, in fact, be misattributing sensations induced by the acetone as symptoms consistent with Gulf War Illness. While brief exposure to low-levels of acetone does not appear to trigger memory loss, learning, or concentration, difficulty sGWVs do appear to have significantly poorer, overall cognitive function as compared to aGWVs.

Studies by Reivich (1974) and Sokoloff (1977) first described the link between regional brain metabolic activity (oxygen utilization) and visual stimulation and motor activity. More recent work confirmed a delicate coupling of regional brain metabolic activity and regional blood perfusion (Lassen and Ingvar, 1990; Leenders et al., 1990; Vanzetta and Grinvald, 1999). Magnetic resonance imaging of the human brain by Belliveau et al. (1991) demonstrated that task-induced neuronal activity is associated with changes in local blood volume. Experimental evidence also indicates that brain metabolic/perfusion coupling is sensitive to a variety of influences including age, trauma, and chemical agents (Vandesteene et al., 1988; Leffler et al., 1989; Balsan et al., 1990; Jakobsen et al., 1990; Marion et al., 1991; Mayevsky and Ziv, 1991; Goldman et al., 1992; Arts et al., 2002).

MCABFV may also reflect cognitive activity. Verbal and visual memory tasks, mental arithmetic, and active participation in commercial video games increase blood flow velocity up to 12% (Droste et al., 1989; Kelley et al., 1992). Consistent with these observations, decreased regional cerebral metabolic rate and blood flow have been related to increasingly severe symptoms in mentally

dysfunctional patients (Gur et al., 1989). Among these conditions, major depression has been associated with a generalized decrease in prefrontal cortex CBF (Drevets, 1998). While sGWVs self-assessed as experiencing more depression than aGWVs, no evidence of reduced CBF that could be attributed to major depression in sGWVs was evident.

The use of TCD for measurement of MCABFV as an estimate of CBF is predicated on the assumption that the insonated vessel maintains a constant diameter throughout the measurement period. Alterations in MCABFV will, therefore, reflect a proportional change in CBF. In this experiment, careful measurements of baseline MCABFVs were made before and at the end of each test condition. The observation that MCABFV under baseline conditions was not changed before or after testing strongly suggests the validity of the assumption of MCA diameter constancy. It also appears that acetone has no effect on MCA vascular tone in either the aGWVs or the sGWVs, in that no change in baseline MCABFV was observed following introduction of acetone into the breathing circuit. As such, the changes in MCABFV in relation to cognitive challenges most probably reflect proportional changes in CBF in the studied subjects.

The idea that organic solvent vapors (acetone) trigger memory loss, difficulty in learning, or confusion is not supported by the findings of this study. However, significant blunting of the CBF response to cognitive challenges following solvent exposure was observed in the sGWVs. Symptoms perceived to be triggered by organic vapor exposure may, in reality, be discomfort-experienced subsequent to the compromised brain calling on its cognitive reserve capacity to meet the challenges. Areas of the brain not normally relegated to specific cognitive activity may be recruited, resulting in a level of discomfort that is misattributed as difficulty in thinking, confusion, or memory loss. This concept may be extended to sGWVs in normal situation (breathing clean air) where CBF response is also not optimal following cognitive challenge.

Cognitive reserve capacity or the degree of unused potential for learning, memory, and executive function that is available to the brain has been shown to decline as a result of various factors such as age, epilepsy, and onset of diseases causing dementia (Salthouse and Lichty, 1985; Baltes et al., 1986; 1992; Raykov, 1989; Schmand et al., 1997; Jokeit and Ebner, 1999). Reaction time, memory performance, and speed of information processing appear to directly reflect the status of the cognitive reserve capacity (Salthouse and Lichty, 1985). Just as other physiological systems' functional reserves are compromised by toxicologic/xenobiotic insults, the brain may also be vulnerable. The diminished cognitive performance and increased task latencies in the sGWVs appear to be consistent with the hypothesis of reduced cognitive reserve capacity.

The mechanism by which the CBF response to cognitive challenge has been altered is still not clear. While the higher resting MCABFV in sGWVs may be the result of higher overall levels of tension and anger, the expected increased blood pressure associated with these elevated moods was not observed. The alternate explanation may suggest an impairment in the ability of the cerebral resistance arterioles to maintain an appropriate vascular tone. The damped elevation in MCABFV during cognitive challenge supports this notion, in that dysfunction in the control of vascular tone may reflect impairment in the perfusion/metabolism coupling mechanism in the sGWVs. Exposure to acetone even at low level appears to dramatically block the elevation of CBF in response to mental activity, suggesting a possible uncoupling of the mechanism.

Metabolic regulation of CBF is a synergistic orchestration of chemical and neurogenic influences. Chemical mediators of vascular tone may no longer be in tune, disrupting the induction of normal decreases in cerebrovascular resistance (CVR) so as to increase CBF in response to cognitive challenge in the sGWVs.

The findings of this study do not demonstrate pulmonary dysfunction in Gulf War veterans who self-assess to having the Persian Gulf Syndrome following exposure to organic solvents. These findings do, however, point to an overall lower cognitive function as compared to aGWVs that worsens with increasingly more difficult or complex cognitive challenges. A more fundamental result of this work suggests a testable hypothesis, namely, that sGWVs may be suffering from a significant reduction in reserve cognitive capacity resulting from a physical dysfunction of the brain's ability to regulate blood flow so as to match local metabolic processes stimulated by cognitive challenges.

Future research should be directed toward further testing of this hypothesis and establishing additional testable hypotheses of Gulf War Illness in order to ultimately develop a theoretical understanding of this condition.

## Acknowledgments

The authors would like to thank Dr Dennis Dobler, Department of Anesthesiology, University of Alabama at Birmingham, for providing Brain Stream Transcranial Doppler blood flow velocity acquisition software.

## References

Anger, W.K., Storzbach, D., Binder, L.M., Campbell, K.A., Rohlman, D.S., McCauley, L., Kovera, C.A. and Davis, K.L. 1999: Neurobehavioral deficits in Persian Gulf veterans: evidence from a population-based study. Portland Environ-

mental Hazards Research Center. *Journal of the International Neuropsychological Society* 5, 203–12.

Anonymous. 1997: Self-reported illness and health status among Gulf War veterans. A population-based study. The Iowa Persian Gulf Study Group. *Journal of the American Medical Association* 277, 238–45.

Arts, J.H., Mojet, J., van Gemert, L.J., Emmen, H.H., Lammers, J.H., Marquart, J., Woutersen, R.A. and Feron, V.J. 2002: An analysis of human responses to irritancy of acetone vapors. *Critical Reviews in Toxicology* 32, 43–66.

Axelrod, B.N. and Milner, I.B. 1997: Neuropsychological findings in a sample of Operation Desert Storm veterans. *Journal of Neuropsychiatry and Clinical Neuroscience* 9, 23–28.

Balsan, M.J., Cronin, C.M., Shaw, M.D. and Milley, J.R. 1990: Blood flow distribution and brain metabolism during tolazoline induced hypotension in newborn dogs. *Pediatric Research* 28, 111–15.

Baltes, P.B., Dittmann-Kohli, F. and Kliegl, R. 1986: Reserve capacity of the elderly in aging-sensitive tests of fluid intelligence: replication and extension. *Psychology and Aging* 1, 172–77.

Baltes, M.M., Kuhl, K.P. and Sowarka, D. 1992: Testing for limits of cognitive reserve capacity: a promising strategy for early diagnosis of dementia? *Journal of Gerontology* 47, P165–67.

Bell, I.R., Warg-Damiani, L., Baldwin, C.M., Walsh, M.E. and Schwartz, G.E. 1990: Self-reported chemical sensitivity and wartime chemical exposures in Gulf War veterans with and without decreased global health ratings. *Military Medicine* 163, 725–32.

Belliveau, J.W., Kennedy, D.N., McKinstry, R.C., Buchbinder, B.R.,Weisskoff, R.M., Cohen, M.S., Vevea, J.M., Brady, T.J. and Rosen, B.R. 1991: Functional mapping of the human visual cortex by magnetic resonance imaging. *Science* 254, 716–18.

Drevets, W. 1998: Functional neuroimaging studies of depression: the anatomy of melancholia. *Annual Review of Medicine* 49, 341–61.

Droste, D.W., Harders, A.G. and Rastogi, E. 1989: Two transcranial Doppler studies on blood flow velocity in both middle cerebral arteries during rest and the performance of cognitive tasks. *Neuropsychologia* 27, 1221–30.

Engel, C.C., Jr., Ursano, R., Magruder, C., Tartaglione, R., Jing, Z., Labbate, L.A. and Debakey, S. 1999: Psychological conditions diagnosed among veterans seeking Department of Defense Care for Gulf War-related health concerns. *Journal of Occupational and Environmental Medicine* 41, 384–92.

Escalante, A. and Fischbach, M. 1998: Musculoskeletal manifestations, pain, and quality of life in Persian Gulf War veterans referred for rheumatologic evaluation. *Journal of Rheumatology* 5, 2228–35.

Fukuda, K., Nisenbaum, R., Stewart, G., Thompson, W.W., Robin, L., Washko, R.M., Noah, D.L., Barrett, D.H., Randall, B., Herwaldt, B.L., Mawle, A.C. and Reeves, W.C. 1998: Chronic multisymptom illness affecting Air Force veterans of the Gulf War. *Journal of the American Medical Association* 280, 981–88.

Goldman, H., Bergman, R.F., Gershon, S., Murphy, S., Morehead, M. and Altman, H.J. 1992: Cerebrovascular permeability and cognition in the aging rat. *Neurobiology of Aging* 13, 57–62.

Goldstein, G., Beers, S.R., Morrow, L.A., Shemansky, W.J. and Steinhauer, S.R. 1996: A preliminary neuropsychological study of Persian Gulf veterans. *Journal of the International Neuropsychological Society* 2, 368–71.

Gray, G.C., Kaiser, K.S., Hawksworth, A.W., Hall, F.W. and Barrett-Connor, E. 1999: Increased postwar symptoms and psychological morbidity among U.S. Navy Gulf. *War Veterans Am J Trop Med Hyg* 60, 758–66.

Gur, R.E., Resnick, S.M. and Gur, R.C. 1989: Laterality and frontality of cerebral blood flow and metabolism in schizophrenia: relationship to symptom specificity. *Psychiatry Research* 27, 325–34.

Haley, R.W. and Kurt, T.L. 1997: Self-reported exposure to neurotoxic chemical combinations in the Gulf War. A cross-sectional epidemiologic study. *Journal of the American Medical Association* 277, 231–37.

Haley, R.W., Kurt, T.L. and Hom, J. 1997a: Is there a Gulf War Syndrome? Searching for syndromes by factor analysis of symptoms. *Journal of the American Medical Association* 77, 215–22.

Haley, R.W., Hom, J., Roland, P.S., Bryan, W.W., Van Ness, P.C., Bonte, F.J., Devous, M.D., Sr., Mathews, D., Fleckenstein, J.L., Wians, F.H., Jr., Wolfe, G.I. and Kurt, T.L. 1997b: Evaluation of neurologic function in Gulf War veterans. A blinded case–control study. *Journal of the American Medical Association* 277, 223–30.

Hudnell, H.K., Otto, D.A. and House, D.E. 1996: The influence of vision on computerized neurobehavioral test scores: a proposal for improving test protocols. *Neurotoxicology and Teratology* 18, 391–400.

Ishoy, T., Suadicani, P., Guldager, B., Appleyard, M., Hein, H.O. and Gyntelberg, F. 1999: State of health after deployment in the Persian Gulf. The Danish Gulf War Study. *Danish Medical Bulletin* 46, 416–19.

Jakobsen, J., Nedergarrd, M., Aarslew-Jensen, M. and Diemer, N.H. 1990: Regional brain glucose metabolism and blood flow in streptozocin induced diabetic rats. *Diabetes* 39, 37–440.

Jensen, T.S. and de Fine Olivarius, B. 1981: Transient global amnesia – its clinical and pathophysiological basis and prognosis. *Acta Neurologica Scandinavica* 63, 20–30.

Jokeit, H. and Ebner, A. 1999: Long term effects of refractory temporal lobe epilepsy on cognitive abilities: a cross sectional study. *Journal of Neurology, Neurosurgery and Psychiatry* 67, 44–50.

Joseph, S.C. 1997: A comprehensive clinical evaluation of 20,000 Persian Gulf War veterans. Comprehensive Clinical Evaluation Program Evaluation Team. *Military Medicine* 162, 149–55.

Kaires, P. 1999: Symptoms in Persian Gulf War veterans. *Journal of Occupational and Environmental Medicine* 41, 939.

Kelley, R.E., Chang, J.Y., Scheinman, N.J., Levin, B.E., Duncan, R.C. and Lee, S.C. 1992: Transcranial Doppler assessment of cerebral flow velocity during cognitive tasks. *Stroke* 23, 9–14.

Kritchevsky, M. 1987: Transient global amnesia. When memory temporarily disappears. *Postgraduate Medicine* 82, 95–97.

Kroenke, K., Koslowe, P. and Roy, M. 1998: Symptoms in 18,495 Persian Gulf War veterans. Latency of onset and lack of association with self-reported exposures. *Occupational and Environmental Medicine* 40, 520–28.

Lassen, N.A. and Ingvar, D.H. 1990: Brain regions involved in voluntary movements as revealed by radioisotopic mapping of CBF or CMR-glucose changes. *Revue Neurologique* 146, 620–25.

Leenders, K.L., Perani, D., Lammertsma, A.A., Heather, J.D., Buckingham, P., Healy, M.J., Gibbs, J.M., Wise, R.J., Hatazawa, J. and Herod, S. 1990: Cerebral blood flow, blood volume and oxygen utilization. Normal values and effect of age. *Brain* 113, 27–47.

Leffler, C.W., Busija, D.W., Mirro, R., Armstead, W.M. and Beasley, D.G. 1989: Effects of ischemia on brain blood flow and oxygen consumption of newborn pigs. *American Journal of Physiology* 257, H1917–26.

Letz, R. 1991: *Neurobehavioral evaluation system 2 (NES2) user's manual (version 4.4)*. Winchester, MA: Neurobehavioral Systems.

Marion, D.W., Darby, J. and Yonas, H. 1991: Acute regional cerebral blood flow changes caused by severe head injuries. *Journal of Neurosurgery* 74, 407–14.

Mattle, H., Edelman, R.R., Wentz, K.U., Reis, M.A., Atkinson, D.J. and Ellert, T. 1991: Middle cerebral artery: determination of flow velocities with MR angiography. *Radiology* 181, 527–30.

Mayevsky, A. and Ziv, I. 1991: Oscillations of cortical oxidative metabolism and microcirculation in the ischaemic brain. *Neurological Research* 13, 39–47.

McCauley, L.A., Joos, S.K., Lasarev, M.R., Storzbach, D. and Bourdette, D.N. 1999: Gulf War unexplained illnesses: persistence and unexplained nature of self-reported symptoms. *Environmental Research* 81, 215–23.

Miller, C.S. and Ashford, N.A. 1999: Chronic multisystem illness among Gulf War veterans. *Journal of the American Medical Association* 282, 328–29.

Miller, C.S. and Prihoda, T.J. 1999: A controlled comparison of symptoms and chemical intolerances reported by Gulf War veterans, implant recipients and persons with multiple chemical sensitivity. *Toxicology and Industrial Health* 15, 386–97.

Mulder, E.J., Linssen, W.H., Passchier, J., Orlebeke, J.F. and de Geus, E.J. 1999: Interictal and postictal cognitive changes in migraine. *Cephalalgia* 19, 557–65.

Norman, G.R. and Streiner, D.L. 1994: Elements of statistical inference: sample size estimation. In Farrell, R., editor, *Biostatistics: the bare essentials*. St. Louis, MO: Mosby, 49–51.

Raykov, T. 1989: Reserve capacity of the elderly in aging sensitive tests of fluid intelligence: a reanalysis *via* a structural equation modeling approach. *Zeitschrift für Psychologie mit Zeitschrift für Angewandte Psychologie* 197, 263–82.

Reivich, M. 1974: Blood flow metabolism couple in brain. In Plum, F., editor, *Brain dysfunction in metabolic disorders*. New York, NY: Raven Press, 125–40.

Roy, M.J., Koslowe, P.A., Kroenke, K. and Magruder, C. 1998: Signs, symptoms, and ill-defined conditions in Persian Gulf War veterans: findings from the Comprehensive Clinical Evaluation Program. *Psychosomatic Medicine* 60, 663–68.

Salthouse, T.A. and Lichty, W. 1985: Tests of the neural noise hypothesis of age-related cognitive change. *Journal of Gerontology* 40, 443–50.

Santoloci, D., Bacigalupo, F., Cocito, L., Farinini, D. and Bo, G. 1985: Transitory global amnesia. *Rivista di Neurologia* 55, 369–76.

Schmand, B., Smit, J.H., Geerlings, M.I. and Lindeboom, J. 1997: The effects of intelligence and education on the development of dementia. A test of the brain reserve hypothesis. *Psychologie Médicale* 27, 1337–44.

Sethre, T., Laubli, T., Berode, M. and Krueger, H. 2000: Neurobehavioral effects of experimental isopropanol exposure. *International Archives of Occupational and Environmental Health* 73, 105–12.

Sillanpaa, M.C., Agar, L.M., Milner, I.B., Podany, E.C., Axelrod, B.N. and Brown, G.G. 1997: Gulf War veterans: a neuropsychological examination. *Journal of Clinical and Experimental Neuropsychology* 19, 211–19.

Sokoloff, L. 1977: Relation between physiological function and energy metabolism in the central nervous system. *Journal of Neurochemistry* 29, 13.

Suadicani, P., Ishoy, T., Guldager, B., Appleyard, M. and Gyntelberg, F. 1999: Determinants of long-term neuropsychological symptoms. The Danish Gulf War Study. *Danish Medical Bulletin* 46, 423–27.

Vandesteene, A., Trempont, V., Engelman, E., Deloof, T., Focroul, M., Schoutens, A. and de Rood, M. 1988: Effect of propofol on cerebral blood flow and metabolism in man. *Anaesthesia. Supplement* 43, 42–43.

Vanzetta, I. and Grinvald, A. 1999: Increased cortical oxidative metabolism due to sensory stimulation: implications for functional brain imaging. *Science* 286, 1555–58.

Weinachter, S.N., Blavet, N., O'Donnell, R.A., MacKenzie, E.T. and Rapin, J.R. 1990: Models of hypoxia and cerebral ischemia. *Pharmacopsychiatry* 23(Suppl 2), 94–97.

West, J.B. 1975: Tests of Pulmonary Function. In West, J.B., editor, *Respiratory physiology – the essentials*. Baltimore, MD: Williams and Wilkins, 146–49.

White, R.F., Diamond, R., Krengel, M., Lindem, K., Feldman, R.G., Letz, R., Eisen, E. and Wegman, D. 1996: Validation of the NES2 in patients with neurologic disorders. *Neurotoxicology and Teratology* 18, 441–48.

Yamane, K., Hashimoto, S., Kobayashi, I. and Maruyama, S. 1989: Basilar artery migraine associated with transient global amnesia. *No To Shinkei* 41, 1103–107.

Zattore, R.J., Jones-Gotman, M. and Rouby C. 2000: Neural mechanisms involved in odor pleasantness and intensity judgments. *NeuroReport* 11, 2711–16.