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Evaluation of cognitive performance in professional divers by means of event-related potentials and neuropsychology

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HIGHLIGHTS

- We investigated the effects of occupational diving on brain functions by ERPs and neuropsychology.
- Some ERP parameters were influenced by diving exposure, and P3 effects were graded by the exposure.
- Extensive diving subgroup displayed poor visuo-construction and visual episodic memory.

ABSTRACT

Objective: We investigated whether professional air diving with no decompression illness causes any long-term changes in cognitive functions.
Methods: The all-male participants consisted of 18 healthy control (HC) volunteers and 32 divers. Divers were divided into two subgroups as moderate exposure group, Divers-I (DI) and extensive exposure group, Divers-II (DII). Participants were administered a comprehensive neuropsychological battery and event-related potentials (ERPs) were recorded while they performed auditory oddball task and visual continuous performance test (CPT).
Results: P3 waves in oddball and CPT were significantly attenuated and peak latencies were prolonged in both diver groups compared with HC. Amplitude decrements in CPT P3 were graded with respect to level of diving exposure. Neuropsychologically, DII group displayed significantly poorer performance than HC and DI groups in measures of visuo-constructional and visual long-term memory tests. DI group performed better than HC group in some measures of planning ability.
Conclusions: Most of the changes in neurophysiological measures and poorer neuropsychological performance were found in DII group, and this might be interpreted as a red flag for the reflection of the slowly progressing deleterious effects of silent bubbles in brain function.
Significance: This study reports impairments in certain neuropsychological measures and apparent neurophysiological markers pointing to slow cognitive decline referring to long-term effects of diving.

1. Introduction

Decompression illness (DCI) is a term used for pathophysiological conditions associated with decompression (Vann et al., 2011; Moon, 1997). While diving, the ambient pressure may drop below the body inert gas tensions during decompression phase (supersaturation), and at a critical point (critical supersaturation) inert gas separates in the bodily fluids and tissues producing gas bubbles. These bubbles may cause local effects or circulate as venous gas emboli. In case of passing through pulmonary or cardiac shunts to the arterial circulation they become arterial emboli (Vann, 2004; Moon, 1999; Blatteau et al., 2006).These inert gas microbubbles, when occurring without any acute clinical signs, historically have been termed 'silent bubbles'(Behnke, 1951), and have been generally assumed to have no negative effects. Although silent bubbles are by themselves asymptomatic, the occurrence of many

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bubbles is clearly linked to a high risk of DCI (Nishi, 1990; Cimsit, 2009).

DCI can affect many systems, its effects on the nervous system are considered to be the most serious (Newton, 2001; Knauth, 2008; Hawes and Massey, 2008; Grønning and Aarli, 2011). Although the relationship between DCI and neurologic damage is obvious, the long-term neurologic effects of diving in divers without history of DCI are far from being unquestionably established. In a recent review by Grønning and Aarli (2011) on the neurological effects of deep-sea diving, the authors concluded that although the immediate and transient neurological effects after deep dives are documented, the results from the epidemiological and clinical studies regarding long-term neurological effects from deep diving are conflicting and still inconclusive.

One observational study reported more general nervous system complaints, most prominent of which were difficulties in concentration and problems with long and short-term memory in professional divers without DCI than controls (Todnem et al., 1990). Cerebral microvascular dysfunction due to gas microembolism is considered as a possible mechanism of long-term neurologic effects (Moen et al., 2010). A number of MRI studies on divers showed increased number of hyperintense white-matter lesions (Tetzlaff et al., 1999; Erdem et al., 2009; Gempp and Blatteau, 2010) and widespread cerebral white matter diffusion changes (Moen et al., 2010) supporting the microvascular dysfunction hypothesis, while others did not (Cordes et al., 2000; Hutzelmann et al., 2000; Koch et al., 2004).

One autopsy study of divers who had died after diving accidents found perivascular lacuna formation in cerebral and/or cerebellar white matter, necrotic foci in grey matter and unilateral necrosis of the head of the caudate nucleus, implicating the bubble as the damaging agent of the cerebral small vessel wall (Palmer et al., 1992). The positive findings suggest that gas microembolism in divers may result in a state similar to that is caused by subcortical ischemic vascular disease (SIVD).

The relationship between SIVD and dysexecutive syndrome is well-established. The executive functions, mediated by prefrontal cortex, include those high level abilities such as abstraction, planning, response inhibition, set-shifting and impairment of these abilities is called dysexecutive syndrome. It has been shown that SIVD, resulting in cognitive impairment, presents with a profile of dysexecutive syndrome (Kramer et al., 2002). The executive dysfunction is also found in asymptomatic individuals with white matter lesions and/or lacunar infarctions (Reed et al., 2004). Another study indicated that regardless of where in the brain these white matter hyperintensities (WMH's) are located, they are associated with frontal hypometabolism and executive dysfunction (Tullberg et al., 2004), supporting the notion that WMHs cause multiple disconnections disrupting the top-down control of the prefrontal network.

The neuropsychological studies on divers with no history of DCI are few in number and their results are somewhat conflicting. Some of these studies point out slight decline in domains such as mental flexibility, inhibition in attentional tasks (Tetzlaff et al., 1999; Slosman et al., 2004), while others report no evidence for such a decline other than longer reaction times (RTs) (Bast-Pettersen, 1999; Cordes et al., 2000). In a study, a mild memory decline was shown by neuropsychological tests on divers with subjective complaint of forgetfulness and this was related with number of dives with gas bounce diving and surface oxygen decompression techniques (Taylor et al., 2006).

Although electroencephalogram (EEG) and event-related potentials (ERPs) provide noninvasive measures for studying brain activity, those methods are rarely used to investigate the long-term neurologic effects of diving. Todnem et al. (1991a) reported focal slow waves in the temporal regions and sharp potentials in 18% of the divers, and these abnormal waveforms were correlated with the exposure to saturation diving and prevalence of decompression sickness. The same group also reported that brainstem auditory evoked potential (BAEP) latencies were increased in the diver groups (Todnem et al., 1991a). However, they reported no abnormal finding in latencies of visual evoked potentials (N75, P100, N145) and brainstem auditory evoked potential, in commercial saturation divers (Todnem et al., 1991b).

In addition to early sensory evoked brain potentials, ERPs with other long-latency brain potentials such as P3 are broadly used to investigate cognitive processes. These brain potentials are well known to be sensitive to several types of cognitive impairments (Polich, 2004), and oddball paradigm has been the most widely recruited cognitive task in these studies. Continuous performance test (CPT) is another widely used cognitive task, and the AX-type CPT involves attention and inhibition systems, which provide basis for executive functions. ERPs during CPT performance are reported to be affected (amplitude decrements and prolonged latency) in certain neuropsychiatric patient groups (Riccio et al., 2002; Fallgatter et al., 2004).

Although our main concern in designing this study was to clarify if prolonged exposure to the extreme conditions as posed by the professional SCUBA (self-contained underwater breathing apparatus) diving had any deleterious effect on cognitive functioning, on the other hand, we were also aware that this profession, which necessitates the development and acquisition of highly specific skills might have resulted with some cognitive gains that could be reflected positively in some neuropsychological test scores and/or in some ERP recording measures.

The aim of this study was to combine a comprehensive neuropsychological test battery (NTB) and ERP recordings in order to investigate the occurrence of likely losses and gains in cognitive functioning in professional air SCUBA divers without history of neurological DCI.

2. Methods

2.1. Participants

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of the Istanbul Faculty of Medicine. All participants gave written informed consent after the aim of the study and the data collection procedures were fully explained to them.

A total of 50 right-handed male volunteers (age range 25–45 years) consented to participate in the study. All participants were in good physical health and none of the participants were taking any medication. Diver groups consisted of professional air SCUBA divers who were employed as diving instructors. Their diving histories, including years of diving experience, total number of dives, average depth (msw), average bottom time (min), deepest dive (msw) and deepest dive time (min) was obtained by a questionnaire. The U.S. Navy Standard Air Decompression Tables were used during the dives. All diver participants had an average depth less than 40 msw and none of them had a history of DCI.

Participants were divided into 3 groups as follows: Healthy Control (HC) = participants with no diving experience ($n = 18$), and divers according to their diving experience: Divers-I Group (DI) = Professional divers with moderate diving experience (1000–2000 dives) ($n = 16$), Divers-II Group (DII) = Professional divers with extensive diving experience (2001–4000 dives) ($n = 16$).

The divers participated to this study following their annual physical examination of head-neck, ear-nose-throat, nervous, respiratory, cardiovascular, gastrointestinal, and genitourinary systems in Department of Underwater and Hyperbaric Medicine, Istanbul Faculty of Medicine.

2.2. Neuropsychological tests

An NTB covering cognitive domains of attention, language, visuo-spatial functions, memory and executive functions was administered to all participants (see Table 1 for the list of neuropsychological tests).

2.2.1. Attention

Digit Span Test (DST) subtest of Wechsler Adult Intelligence Scale. On this test, the participant is required to repeat a mixed array of digits, first in the same order (forward span) and then in the reverse (backward span) order. The former is considered as a measure of global attention, whereas the latter is associated with working memory (Wechsler, 1981).

2.2.2. Language

Boston Naming Test (BNT) is a test of confrontational naming, it contains line drawings of natural and artificial objects graded in word frequency (Kaplan et al., 1983).

2.2.3. Visuo-spatial functions

These are traditionally classified as visuo-perceptual and visuo-constructive tasks. Whereas visuo-perceptual tasks are considered as purer measures of right hemispheric visuo-spatial ability, visuo-constructional tasks are thought to contain an executive element as the construction with manipulation within a time limit entails efficient planning (Weintraub, 2000).

Benton's Line Orientation (BLO) test is a visuo-perceptual task, as it depends solely on visual analysis for matching angles (Benton et al., 1998).

Block Designs Test (BDT), a subtest of Wechsler Adult Intelligence Scale-Revised (Wechsler, 1981) is a visuo-constructional task that needs manipulation and visual analysis. Two-dimensional drawing of a design must first be visually inspected, and then, the same design must be constructed with 4 or 9 cube blocks within a certain time limit.

Copying Rey-Osterrieth Complex Figure test (ROCFT-Copy) is also a measure of visuo-constructive ability. First examinees are asked to reproduce a complicated line drawing by copying the original figure and the performance is scored (Loring et al., 1990).

2.2.4. Verbal memory

California Verbal Learning Test (CVLT) is a word-list learning test in which the subject is required to learn as many words as possible from a total of 16 words for 5 trials. That list of 16 words is read to the subject in a predefined random order, but the words actually belong to 4 different semantic categories (i.e., spices, fruits, tools, garments) by 4-word clusters. Short-delay free and cued-recall trials have 4 category names given as cues that come after the single presentation of similarly structured but a different word list (B list). After a 20-min delay, during which the subject performs non-verbal tasks, there are the long-term free and cued-recall trials. The test is concluded with a recognition trial where the 16 target words are scattered among 28 distracters (Delis et al., 1987).

2.2.5. Visual memory

ROCFT immediate recall (IR) and delayed recall (DR) are measures of visual memory. ROCFT-IR is the immediate reproduction from memory of the previously copied complex figure and ROCF-DR is the delayed recall of the same figure after 30 min.

2.2.6. Executive functions

Wisconsin Card Sorting Test (WCST) is a test of mental set shifting, in which the subject is required to sort 2 decks of cards under the reference cards according to pre-specified rules unbeknownst to him/her, and also, the subject is not told that the current rule changes after 10 correct sortings. The successful subject should not "perseverate" with the old rule and using the negative feedback from the tester should infer the new rule. The efficiency of the examinee becomes the index of mental flexibility (Heaton, 1981).

Tower of London Test (ToL) is a task of planning ability in which the subject has to solve positional problems by moving 3 colored beads one at a time among 3 sticks with different heights. The problem must be solved by starting from an initial position and finishing at a pre-specified end-position with a minimum number of moves and within the shortest time. The necessary moves must be planned in advance and executed as fast as possible within the permitted rules (Culbertson and Zillmer, 2005).

Verbal Fluency Tests (semantic and lexical fluency) are measures of sustained attention. In this particular version, subjects are asked to name as many animals as possible within 1 min (semantic fluency) and as many words as possible that begin with letters K, A, and S within 1 min for each letter (lexical fluency) (Spreen and Strauss, 1998).

Stroop Test includes a task for assessing complex selective attention and interference control as an executive function. The interference task of the test consists of the color words that are incongruently colored than what the word denotes. The subject is required to name the color of the word while trying to inhibit the interference of the automatic response tendency of reading the word instead (Spreen and Strauss, 1998).

2.4. ERP recordings

The EEG was recorded in an electrically shielded, sound-attenuated and dimly illuminated room. Participants were seated in a comfortable armchair, and the EEG signal was collected using Ag/AgCl ring electrodes mounted in an elastic cap (*EasyCap*,

Table 1
Neuropsychological tests battery.

Cognitive domain	Neuropsychological tests
Attention	Digit Span Test (DST)
Language	Boston Naming Test (BNT)
Visuo-spatial functions	Benton's Judgment of Line Orientation Test (BJLOT)
	Block Design Test (BDT)
	Rey-Osterrieth Complex Figure Test (ROCFT) - Copy
Memory	California Verbal Learning Test (CVLT)
	Rey-Osterrieth Complex Figure Test (ROCFT)- Immediate and Delayed Recall
Executive functions	Wisconsin Card Sorting Test (WCST)
	Tower of London Test (ToL)
	Stroop Test
	Verbal Fluency Tests (VFT) - (Category and 3-letter fluency)

Herrsching, Germany) from 30 channels (Fp1, Fp2, Fz, F3, F4, F7, F8, FCz, FC3, FC4, FT7, FT8, Cz, C3, C4, T7, T8, CPz, CP3, CP4, TP7, TP8, Pz, P3, P4, P7, P8, Oz, O1, O2) according to the extended international 10/20 placement system. Electro-oculogram (EOG) was recorded bipolarly from cup electrodes placed at the outer canthus of the right eye and nasion in order to trace the eye movement related artifacts on EEG. All EEG activity was referenced to linked earlobes. Electrode impedances were kept below 10 kΩ. EEG and EOG were amplified by *La Mont, 32-Ch EEG Type II* (La Mont Medical Inc., Madison, WI, USA) with a band pass filter of 0.1–70 Hz and digitized with a sampling rate of 200 Hz.

ERPs were recorded while the participants performed an auditory oddball task and a visual continuous performance test (CPT). Stimulus presentation was carried out using a MATLAB program developed using the Psychophysics Toolbox (Brainard, 1997).

The auditory stimuli were of a set of 300 tones in which the standard tones (1000 Hz, 70 dB, 50 ms duration) occurred on 80% of the trials and the target tones (1500 Hz, 70 dB, 50 ms duration) occurred on 20% of the trials. The participants were instructed to respond with button press when the target tone was presented. The tones were presented in a random fashion with an inter-stimulus interval of 2000 ms.

The CPT was performed with a sequence of 400 visual stimuli. All stimuli were singly presented for 200 ms with an inter-stimulus interval of 1500 ms on the center of a 15 inch computer screen as white letters of 4.5×4.5 cm over a gray background from a distance of 80 cm with a visual angle of 3°15 ▫ both horizontally and vertically. The stimuli set was consisted of a primer "A", a conditional target "Z", and 10 distracters (B, C, D, E, F, G, H, J, K, L) appearing in random order with the following condition probabilities: 20% for the primer ("A"), 10% for Go stimuli ("Z" preceded by the primer, "A"), 10% for NoGo stimuli (any distracter letter followed by the primer, "A"), and 60% for the remaining distracter stimulus conditions.

In both ERP tasks, the participants were requested to give equal emphasis on speed and accuracy. In order to evaluate the behavioral performance, the RTs of the hits (the latency of correct mouse-clicks), the commission error score (the number of incorrect mouse-clicks for the CPT/NoGo stimulus) and omission score (the number of missed mouse-clicks for oddball/target or Go stimuli) were recorded over the offline stored text data.

MATLABR2010b software package was used for EEG data analyses. EEG was epoched between 500 ms pre-stimulus and 1000 ms post-stimulus time windows. Single trials with EEG or EOG amplitudes exceeding ±90 μV were rejected automatically as artifact. The remaining EEG epochs were examined and trials with visible artifacts were manually rejected. Epochs were then baseline corrected to pre-stimulus 200 ms before ERP evaluation. We designated the following waves and post-stimulus time windows to be evaluated by inspection of the grand averaged ERPs (Fig. 1) as follows: N1 (70–120 ms), P2 (150–250), N2 (200–300 ms), P3 (250–450 ms).

2.5. Statistical analyses

2.5.1. ERPs

ERP data from 9 channels (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4) were included in the statistical analyses of N1, P2, N2, P3 waves obtained by auditory oddball task. The occipital channels (O1, Oz, O2) were added to set of 9 channels for P3 analyses of the CPT data. The N2 wave in CPT data was analyzed in NoGo condition from fronto-central electrodes (F3, Fz, F4, C3, Cz, C4) where this waveform was observed (see waveforms from midline electrodes in Fig. 1). The differences in amplitude and latency of ERP peaks were analyzed using a repeated measures ANOVA design with group factor (GROUP: HC, DI, DII) as the between subjects factor, antero-posterior topography (AP: frontal, central, parietal, occipital) and lateral topography (LAT: left, midline, right) factors as within subject factors. Number of AP factor levels depended on the electrodes included in the analyses: oddball N1, P2, P3 waves consisted of 3 levels (frontal, central, and parietal); the CPT P3 wave consisted of 4 levels (frontal, central, parietal, and occipital) and the CPT N2 wave consisted of 2 levels (frontal, central). ERP data from each task condition were analyzed separately. Post-hoc analysis with *Bonferroni* correction is applied for pairwise group comparisons. *Greenhouse–Geisser* correction procedures were applied to the degrees of freedom when the repeated measure factor contained more than two levels.

2.5.2. Univariate analyses

Demographic characteristics, neuropsychological measures, RTs and correctness scores of ERP task performance were analyzed as follows: Normal distribution was checked by Shapiro–Wilk test and homogeneity of variance was checked by Levene test in measures from each group. If normality was justified ($p > 0.05$ in Shapiro–Wilk test) one-way ANOVA test was carried out with Bonferroni corrected pair-wise group comparisons. Welch F-ratio is reported when the normally distributed data did not meet assumptions for homogeneity of variance ($p > 0.05$ in Levene test), followed by Games-Howell tests for post hoc comparisons. When normality was violated, Kruskal–Wallis test with Dunn–Bonferroni post-hoc comparisons was used.

3. Results

3.1. Demographic characteristics and diving history of the participants

Means of total number of dives were 1740 ± 263 in DI group (1000–2000 dives) and 3480 ± 639 in DII group (2000–4000 dives). Age and education (year) of the three groups were comparable with no statistically significant differences. DII group had significantly more diving experience (year) ($F(1,18.13) = 31.909$, $p < 0.001$) and depth of deepest dive (msw) compared with DI group ($F(1,30) = 10.946$, $p < 0.01$). The deepest dive time (min) was significantly higher in DI group ($p < 0.05$). There was no significant difference between the diver groups for average depth (msw) and average bottom time (min). The demographic characteristics and diving history of the participants are presented in Table 2.

3.2. Behavioral performance in ERP tasks

There was a significant group effect on RTs in the oddball ($F(2,47) = 4.650$, $p < 0.05$) and CPT ($F(2,27.48) = 8.043$, $p < 0.01$, see Table 3). Mean RTs of both diver groups were approximately 60 ms longer than the mean RTs of HC group. Post-hoc tests confirmed RT prolongation in DI and DII groups compared with HC group in the oddball task ($p < 0.05$ for both comparisons) and CPT (DI vs. HC, $p < 0.05$; DII vs. HC, $p < 0.01$). There was no significant difference in RTs between DI and DII groups. The omission scores and commission error scores yielded no significant group effect (see Table 3).

3.3. ERPs

In the oddball target condition, there was a significant group effect on N1 amplitude ($F(2,47) = 4.164$, $p < 0.05$) and post hoc comparisons revealed that N1 peak was less negative (i.e. smaller amplitude) in DII group than in HC group ($p < 0.05$). There was also a significant group effect on target P2 amplitude ($F(2,47) = 4.280$, $p < 0.05$), and target P2 amplitude in DII group was larger than in

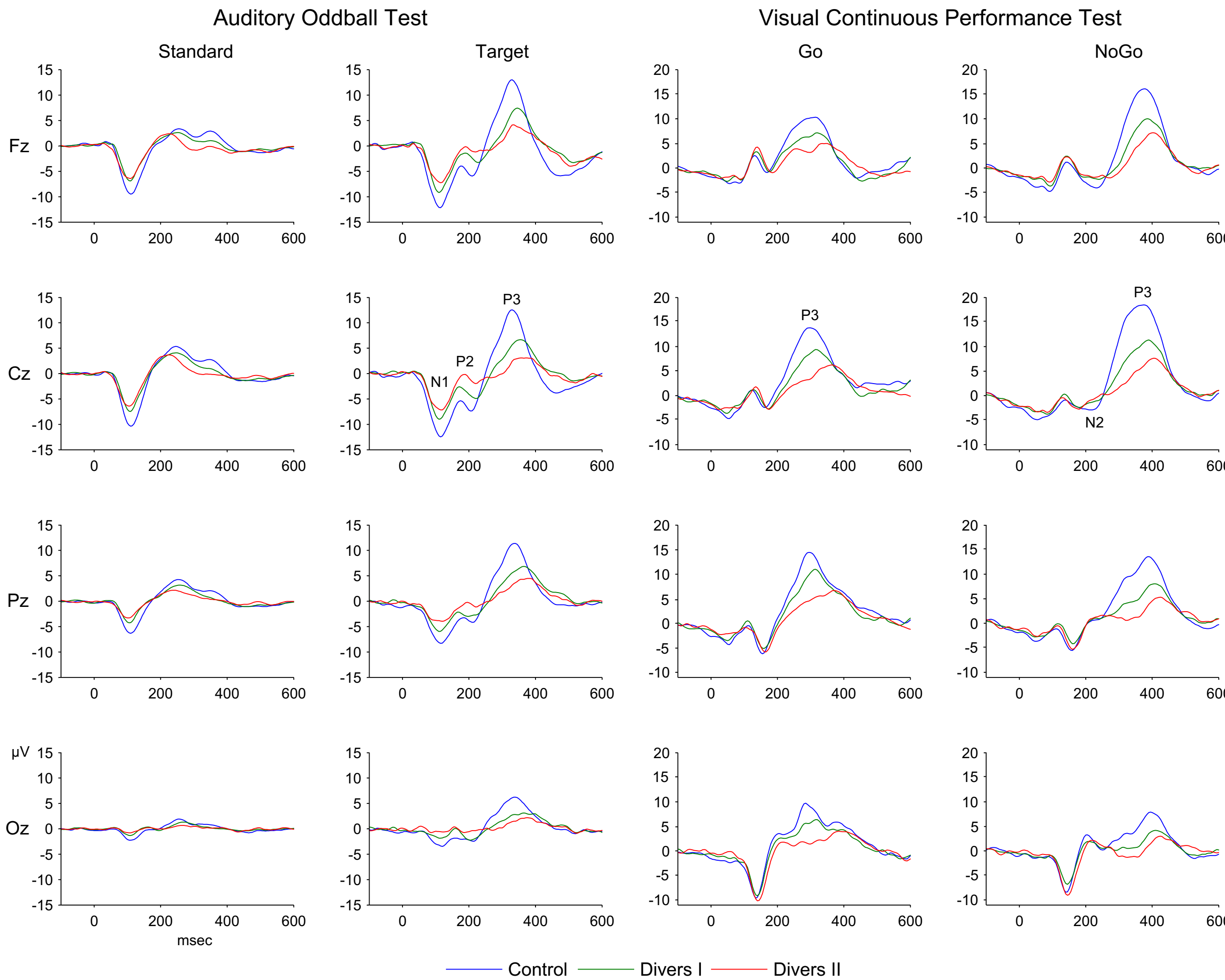

Fig. 1. Grand average ERPs from midline electrodes.

Table 2
The results of demographic characteristics and diving history of the groups.

Participants	HC	DI	DII	*df*	*F*	*p*	η_p^2
Age (yr)	34.8 ± 5.1	34.6 ± 5.1	35.3 ± 5.4	2/47	0.07	0.933	–
Education (yr)	14.9 ± 3.4	14.7 ± 2.9	14.3 ± 3.0	2/47	0.150	0.861	–
†Diving experience (yr)	–	12.8 ± 1.6	20.1 ± 5.2	1/18.13	31.909***	<0.001	0.515
Average depth (msw)	–	33.0 ± 5.9	36.3 ± 8.5	1/30	1.754	0.195	–
Average bottom time (min)	–	22.2 ± 4.1	22.8 ± 5.9	1/30	0.126	0.726	–
Deepest dive (msw)	–	54.1 ± 10.2	65.6 ± 15.9	1/30	7.817**	0.009	0.207
Deepest dive time (min)	–	20.6 ± 5.8	15.0 ± 6.1	1/30	10.946**	0.002	0.267

HC: Healthy Controls; DI: Divers-I; DII: Divers-II.
** $p < 0.01$; *** $p < 0.001$; †Welch's ANOVA result is reported.

HC group ($p < 0.05$). Target N2 amplitude and N2 latency measures yielded significant overall group effects (F (2,47) = 3.987, $p < 0.05$ and F (2,47) = 7.140, $p < 0.01$, respectively). Post-hoc tests showed that DII group had significantly smaller N2 amplitude ($p < 0.05$) and longer N2 latency ($p < 0.01$; Fig. 1) than HC group.

Analyses of target P3 amplitude and P3 latency showed significant group effects (F (2,47) = 12.833, $p < 0.001$ and F (2,47) = 4.670, $p < 0.05$, respectively), subsequent post-hoc group comparison results are as follows: P3 amplitudes in both DI and DII groups were significantly smaller than that of HC group ($p < 0.05$ and $p < 0.001$, respectively). There was also a strong trend for smaller P3 amplitude in DII group compared with DI group ($p = 0.067$). P3 latency in DII group was longer than that of HC group ($p < 0.05$). The grand averaged standard (non-target) oddball stimuli ERPs showed smaller N1 amplitude in divers (see Fig. 1), and this effect was not found to be significant (see Table 4).

In the CPT/Go condition, we found significant group effects on P3 amplitude (F (2,47) = 44.104, $p < 0.001$) and P3 latency (F (2,47) = 18.861, $p < 0.001$). Post-hoc group comparisons revealed that P3 amplitude in DI and DII groups were smaller than in HC group ($p < 0.001$ for both comparisons), and P3 amplitude in DII group was also lower than in DI group ($p < 0.001$). P3 latency in

Table 3
The results of behavioral performance in ERP paradigms.

Performance variables	HC	DI	DII	df	Test statistics[‡]	p	η_p^2
Oddball task							
[A]Reaction time (ms)	328.7 ± 36.2	386.4 ± 80.5	384.5 ± 67.4	2/47	4.650[*]	0.014	0.165
Omission score	0	0	0	2	3.100	0.212	–
Commission error score	0	0.5	0	2	0.607	0.738	–
CPT							
[†,A]Reaction time (ms)	322.9 ± 37.3	376.8 ± 68.6	386.0 ± 63.2	2/27.48	8.043[*]	0.002	0.206
Omission score	0	0	0	2	1.481	0.477	–
Commission error score	0.625	1	1	2	2.145	0.342	–

HC: Healthy Controls; DI: Divers-I; DII: Divers-II.
‡ Kruskal-Wallis test is carried out and χ^2 test with medians are reported unless a footnote appears at the name of the parameter.
A One-way ANOVA is carried out and F ratio is reported with means and standard deviations.
† Welch's ANOVA result is reported.
* $p < 0.05$.

Table 4
The results of ERP amplitude and latency in the oddball and CPT.

	ERPs	df	Amplitude		η_p^2	Latency		η_p^2
			F	p		F	p	
Oddball/Standard	N1	2,47	1.851	0.168	–	0.692	0.506	–
Oddball/Target	N1	2,47	4.164[*]	0.022	0.151	0.615	0.545	–
	P2	2,47	4.280[*]	0.020	0.154	2.287	0.113	–
	N2	2,47	3.987[*]	0.025	0.145	7.140[**]	0.002	0.233
	P3	2,47	12.833[**]	0.001	0.353	4.670[*]	0.014	0.166
CPT/Go	P3	2,47	44.104[***]	<0.001	0.652	18.861[***]	<0.001	0.445
CPT/NoGo	N2	2,47	0.911	0.409	–	1.778	0.180	–
	P3	2,47	38.462[***]	<0.001	0.621	6.112[**]	0.004	0.206

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

DII group was significantly longer than both HC and DI groups ($p < 0.001$ for both comparisons; Fig. 1).

There was also significant group effects on NoGo P3 amplitude and latency (F (2,47) = 38.462, $p < 0.001$ and F (2,47) = 6.112, $p < 0.01$, respectively). Post-hoc group comparisons showed that NoGo P3 amplitudes in both DI and DII groups were lower than HC group ($p < 0.001$ for both comparisons). NoGo P3 amplitude in DII group was also smaller than that of DI group ($p < 0.05$). NoGo P3 latency in DII group was significantly longer than in HC group ($p < 0.05$; Fig. 1). There was no significant group effect on fronto-central N2 amplitude and latency which was evaluated in the CPT/NoGo condition.

Topographic distribution of the ERPs did not yield any significant group effects. See Table 4 for summary of the ERP results.

3.4. Neuropsychological Testing results

BDT performance was significantly different among the groups ($\chi^2(2) = 6.468$, $p = 0.039$). The post hoc comparisons revealed that this resulted from the poorer performance of DII group compared with HC group ($p < 0.05$). ROCFT-Delayed Recall Performance (DRP) was significantly different among the groups (F (2,30.55) = 8.185, $p < 0.01$). DII group performed poorer than both HC ($p < 0.05$) and DI ($p < 0.01$) groups, and there was no difference between DI and HC shown by post hoc comparisons. The ROCFT-Copy and –Immediate Recall scores were not different among the groups.

ToL performance yielded several significant group effects. ToL-Total Correct Score (ToL-TCS) was significantly different among the groups ($\chi^2(2) = 8.114$, $p < 0.05$). The post hoc comparisons revealed that DI group had performed better than HC ($p < 0.05$). ToL-Total Move Score (ToL-TMS) was also significantly different among the groups (F (2,28.76) = 4.306, $p < 0.05$). The post-hoc comparisons revealed that HC group needed more moves in order to complete ToL problems compared with DI group ($p < 0.05$).

There was a trend effect for planning time to initiate first move in ToL (ToL-TIT) score ($\chi^2(2) = 4.944$, $p = 0.084$. The medians of the groups suggests a longer initiation time in DI group compared to two groups.

There were no statistically significant differences among the groups regarding any other neuropsychological measures (i.e. the remaining neuropsychological tests; DST, BNT, BJLOT, CVLT, WCST, VFT, ST). See Table 5 for the summary of the NTB results.

4. Discussion

There has been a debate on whether repeated diving may cause long-term effects on the nervous system even in divers who have not experienced DCI (Tetzlaff et al., 1999; Cordes et al., 2000; Reul et al., 1995). Todnem et al. (1990) compared 156 air and saturation divers with 100 controls. The same group study also examined 40 saturation divers 1–7 years after their last deep dive and 100 controls (Todnem et al., 1991b). They found similar results such as more memory problems, difficulties in concentration and neurological findings on clinical examination and abnormal EEG (Todnem et al., 1991a). The general neurological symptoms and findings had independently significant correlations with diving exposure, prevalence of DCI and age.

We used ERPs and NTB to investigate the possible cognitive impairments associated with the exposure in occupational air SCUBA divers without history of neurological DCI. All participants were diving instructors and the main criterion for setting the two diving exposure indices was number of dives (at least 1000 dives), which naturally accompanied with the period of occupational diving. The two diving groups were homogeneous in average diving conditions (average depth, average bottom time), though

Table 5
The results of neuropsychological tests battery.

	HC	DI	DII	*df*	Test statistics[‡]	*p*	η^2
Digit Span Test-forwards	7	6.5	6	2	4.071	0.131	–
Digit Span Test-backwards	6	6	5.5	2	2.125	0.346	–
Boston Naming Test	31	31	31	2	3.835	0.147	–
Block Design Test	40.5	37	30.5	2	6.468*	0.039	0.053
[A]BJLOT	26.1 ± 3.2	26.3 ± 3.0	25.6 ± 2.3	2/47	0.238	0.789	–
ROCFT- Copy	32	30.5	29.5	2	4.973	0.083	–
[A]ROCFT-IRP	22.4 ± 5.4	23.1 ± 3.7	20.1 ± 5.1	2/47	1.748	0.185	–
[†,A]ROCFT-DRP	24.1 ± 6.7	23.6 ± 3.3	19.1 ± 3.6	2/30.55	8.185**	0.001	0.183
CVLT-1st trial	7	7	7.5	2	2.358	0.308	–
CVLT-total trials	57.5	53.5	53.5	2	3.073	0.215	–
CVLT-SDFR	13	13	12	2	0.255	0.880	–
[†,A]CVLT-SDCR	13.7 ± 1.9	13.1 ± 1.4	12.4 ± 2.6	2/29.55	1.227	0.308	–
CVLT-LDFR	12.5	14	13.5	2	1.499	0.473	–
CVLT-LDCR	14	14	13	2	0.268	0.856	–
CVLT-recognition hits	16	15	16	2	1.361	0.506	–
ToL-TIT (sec)	22	41	33.5	2	4.944	0.084	–
ToL-TCS	4	6.5	4.5	2	8.114*	0.017	0.087
[†,A]ToL-TMS	28.6 ± 10.3	18.6 ± 11.7	32.0 ± 20.8	2/28.76	4.306*	0.023	0.132
ToL-TET (sec)	147	137	161.5	2	1.499	0.473	–
Stroop-interference time	27.1 ± 11.0	27.7 ± 10.0	33.6 ± 12.9	2/30.77	1.384	0.266	–
[†,A]VFT-animals	30.0 ± 9.1	32.9 ± 7.5	30.2 ± 7.1	2/31.30	0.725	0.492	–
[†,A]VFT- letter K	21.3 ± 7.9	20.3 ± 5.8	18.6 ± 5.5	2/31.20	0.754	0.479	-
VFT-letter A	15	15	13.5	2	1.398	0.497	–
VFT-letter S	17	16	11	2	4.662	0.097	–
WCST-PR (%)	8	6	8	2	1.896	0.387	–
WCST-total categories	6	6	6	2	0.979	0.613	–

BJLOT: Benton's Judgment of Line Orientation Test; ROCFT: Rey-Osterrieth Complex Figure Tests, -IRP: Immediate Recall Performance, -DRP: -Delayed Recall Performance; CVLT: California Verbal Learning Test, -SDFR: -Short Delay Free Recall, -SDCR: Short Delay Cued Recall, -LDFR: Long Delay Free Recall, -LDCR: Long Delay Cued Recall; ToL: Tower of London Test, -TIT: Total Initiation Time, -TCS: Total Correct Score, -TMS: Total move score, -TET: Total Execution Time; VFT: Verbal Fluency Test; WCST: Wisconsin Cart Sorting Test, -PR: Perseverative Response, HC: Healthy Controls; DI: Divers-I; DII: Divers-II. * $p < 0.05$; ** $p < 0.01$.

‡ Kruskal-Wallis test is carried out and χ^2 test with medians are reported unless a footnote appears at the name of the neuropsychological test parameter.

A One-way ANOVA is carried out, *F* ratio is reported with means and standard deviations.

† Welch's ANOVA result is reported.

there were some small differences between rare peak experiences (i.e. depth of deepest dive, deepest dive time). As in most of the studies, the diving experience was obtained by questionnaires based on divers' recall. Questionnaires remain as the necessary tool for diving experience because most divers disregard complete filling of their diving-log or they don't bring these logs to their appointments. In the present study, the diver participants were tightly instructed to be as accurate as possible during the questionnaires and their answers were checked for consistency between several applications to minimize the inevitable imprecision in diving experience measures.

ERPs obtained by oddball task (a simple auditory stimulus discrimination task) and CPT (a visual sustained attention test) yielded significant differences between the three participant groups in many ERP parameters including N1, P2, N2, P3 amplitudes and/or latencies. Most of those differences in ERPs were found between DII group (extensive diving exposure) and HC group (healthy non-divers).

Both ERP tasks required responding by button press to the infrequent target stimulus (oddball/target and CPT/Go stimulus conditions). The longer RT findings in both DI and DII are somewhat coherent with several previous prolonged RT findings during neuropsychological tests (Tetzlaff et al., 1999; Slosman et al., 2004; Bast-Pettersen, 1999; Cordes et al., 2000). Contrary to these findings, Hemelryck et al. (2014) reported shorter RTs in simple reaction times of a recreational diver group compared with controls. However, in that study the mean diving experience of the recreational divers was markedly less than the diver participants recruited in the present study and previous studies that have reported longer RTs in divers.

P3 is one of the most widely investigated ERP component in several populations, and its attenuation is repeatedly reported in normal aging and several neurological and psychiatric populations including Alzheimer's disease, Parkinson's disease, depression and schizophrenia (Polich et al., 1990; Polich, 1997; O'Donnel et al., 1987; Blackwood et al., 1987). When P3 is obtained in response to processing target stimuli interspersed among non-targets, it displays typical scalp topography with centro-parietal maximum, termed as P3b. This waveform is suggested to involve activation of event-categorization network by cooperative involvement of attention and working memory (Kok, 2001; Polich, 2007). The intracortical recordings showed that P3b is accompanied by activation in widespread cortical areas including ventral temporofrontal cortices, superior temporal sulcus, posterior parietal cortex and the hippocampus (Halgren et al., 1998) with significant contribution of parietal cortex to the waveform recorded from scalp.

The CPT/Go condition is somewhat comparable to oddball target condition in terms of behavioral response execution. Accordingly, we observed that the CPT/Go P3 displayed the typical P3b topography with centro-parietal maxima which was similar to previous reports on CPT/Go P3 topography (Fallgatter et al., 2002; Kirmizi-Alsan et al., 2006).

The current study clearly showed significant P3b attenuation in the SCUBA diver groups compared with non-diver controls in oddball and CPT. Moreover, in the CPT, the Go P3 attenuation was significantly graded with respect to the diving experience, i.e., P3 amplitudes were graded as HC > DI > DII. Thus, CPT ERPs proved to be sensitive for showing the differences in brain activity between moderate and extensive diving exposure groups (DI and DII). Oddball P3 also showed the similar graded attenuation, but the difference between DI and DII was at trend level. This superior sensitivity of CPT can be attributed to task complexity and the cognitive demand that involves certain executive functions like response inhibition, resistance to interference. In the CPT, all stimuli are evaluated according to the preceding stimulus, involving more of working memory and anticipation after cue stimulus

("A" letter) while oddball stimuli are evaluated singly independent of the preceding stimulus.

In the response execution conditions (oddball/target and CPT/Go), although there was no significant difference in correctness scores, both RTs and P3 latencies were prolonged in DII group suggesting a relatively slow processing. P3 latency prolongation is reported in normal aging, several neurological and psychiatric patient groups (Polich and Herbst, 2000), including patients with SIVD (Muscoso et al., 2006). RTs of DI group were also prolonged but P3 latency prolongation was not significant in that diver group. An extensive review on the utility of P3 latency, postulated a partial relation between RT and P3 latency, and argued overlapping of stimulus evaluation-related and response-related cognitive processes during P3 generation (Verleger, 1997). Based on this theory, in DII group both stimulus evaluation and response-related components were affected. On the other hand, comparable P3 latency between DI group and HC groups suggests that timely activation of stimulus evaluation-related circuitry remained mostly unaffected in DI group.

In the CPT/NoGo trials where button press appealed by the cue stimulus was to be withheld, P3 displayed larger amplitude with anterior localization compared with the CPT/Go P3. The anterior location and large amplitude of NoGo P3 are well-reported features in the previous studies (Bokura et al., 2001; Fallgatter et al., 2002). In DI and DII groups NoGo P3 amplitudes were smaller, and P3 latency was prolonged in DII group that are implicates of a distress in inhibition of the inappropriate motor response. NoGo attenuation in CPT is mostly reported in patient groups suffering from response control and inhibition, such as unmedicated attention deficit hyperactivity disorder (ADHD) (Fallgatter et al., 2004). The source localization of CPT NoGo indicates strong electrical activity of the anterior cingulate cortex (ACC; Brodmann Area 24) which is one of the most pronounced brain structure related with response inhibition (Fallgatter et al., 2002).

We hypothesized that a likely cognitive impairment associated with diving might be due to the cumulative effects of silent bubbles involving penetrating small arteries and arterioles. The full-blown cognitive profile associated with such an involvement is expected to be a dysexecutive syndrome. Prefrontally mediated executive functions are prone to deterioration not only by lesions involving the frontal lobes per se, but also by widespread subcortical lesions, as they disconnect frontal lobes from their reciprocally interconnected discrete neurocognitive networks (Tullberg et al., 2004). Although the commission error scores (behavioral indicative of response inhibition) of both diver groups were comparable to HC, the diminished and delayed CPT/NoGo P3 in the divers can be a marker of vulnerability to slowly progressing subtle executive dysfunction in line with the silent bubble hypothesis.

The pre-P3 waves, N1, P2 and N2 are associated with the cognitive processes of selective attention (Luck et al., 2000). In the oddball/target ERPs, we found that amplitudes of N1, P2 and N2 were remained more positive in DII group (smaller N1 and N2, larger P2) compared with HC group. N1 enhancement to attended target auditory stimulus, compared with the frequent non-target stimulus is an established brain response designating selective auditory attention (Hansen and Hillyard, 1980), seemed to be lacking in DII group. P2 wave is suggested to index some aspect of orienting response, early stimulus classification process, and amplitude of this wave is expected to decrease when stimulus is the attended infrequent target (Crowley and Colrain, 2004). In the present study, diver ERPs displayed smaller N1 (less negativity) and higher P2 amplitude in response to oddball/target stimuli implying poor attention allocation to the target. In the attended auditory stimulus conditions, these two parameters N1-P2 is also interpreted as a single complex, the negativity shift overlapping both N1 and P2 in response to deviant target auditory stimuli, termed as processing negativity appearing as enhanced N1 (more negative) and suppressed P2 (less positive) (Michie et al., 1990). N2 wave is a negative ERP that also occurs in task conditions like oddball target, where the participant deliberately focuses on the deviant stimuli (Näätänen, 1990). Therefore, smaller N1, larger P2 and smaller N2 in oddball target in DII group, collectively suggests attenuation in selective attention to targets, that is assumed to act as a preceding filter to guide the following decision stages.

In summary of ERP results, P3 attenuation was the most sensitive parameter in revealing the possible changes in brain function of both diver groups. Also, P3 amplitudes of the CPT were even sensitive to the difference between DI and DII, showing more attenuation with increased exposure to SCUBA diving. The CPT/Go P3 latency also indicated susceptibility to slower processing to act in DII group not only compared with HC but also DI. The following ERP parameters were different between non-diver controls and DII but not DI: N1 amplitude, P2 amplitude, N2 amplitude and latency, P3 latency of oddball/target and P3 latency of CPT/NoGo trials.

Rather than the somewhat graded negative findings implicating deleterious effects of diving experience as a function of exposure, neuropsychologically we found no losses but specific gains in DI group and hemispherically lateralized losses in DII group.

A successful problem solving during the ToL test entails a mental rehearsal of the problem, during which the participant tries to visualize the minimum necessary moves between the start and end-positions of the beads. ToL-TCS is a straightforward index of efficient planning ability as it is the number of correctly solved problems. In the present study, DI group solved more problems correctly (higher ToL-TCS) as compared with both the non-diver HC. ToL-TMS refers to the total number of moves required to accomplish the test. DI group ToL-TMS score was less than HC group as indicative of better performance. We think that these findings indicating a better planning ability in DI group may fulfill our second expectation and interpreted as the cognitive advantage for an individual after acquiring a complex skill such as diving. One is tempted to think that acquisition of this skill would have endowed a diver with enhanced planning and problem-solving skills, crucial for acting and navigating in a potentially dangerous environment. Yet this advantage could be U-shaped and lost after passing the threshold for deleterious effects of long-term diving. Hence, ToL measures of control participants and extensive divers (DII) were comparable.

The deleterious effect of diving on neuropsychological functioning was only reflected by the poorer performance of the more extensive divers in DII group on ROCF-DRP and BDT compared with the HC group. Moderate divers (DI group) performed comparable to the non-divers in ROCF-DRP and scored significantly better than their diver colleagues with history of more extended diving. Their performance on BDT stayed in between the other two groups.

ROCF-DRP requires copy drawing a complex figure after a delay period of 20–30 min. It is an index of the integrity of structures devoted to visual episodic memory mainly located in the right hemispheric limbic structures (Spreen and Strauss, 1998). Likewise, BDT score is an index of visuo-constructional functions mediated by right hemispheric heteromodal cortices (Benton et al., 1998). Besides, verbal memory functions known to be associated with left hemisphere structures were not impaired. A straightforward conclusion cannot be drawn based on this evidence implicating a right hemisphere vulnerability to the long-term negative effects of diving. Yet it may be speculated that human perceptual, motor and cognitive skills that have evolved to adapt to terrestrial conditions do not suit underwater conditions, which is not a natural habitat for humans. It can be considered as an extreme condition for which special adaptations of those skills are needed and long-term exposure to this extreme condition may well affect those skills differentially. Underwater milieu presents a radically different visual environment for human species. This distinct visual environment would entail adaptive plastic changes in the visual cortices constructing sensory representations and in the structures devoted to maintain these visual representations. The brain structures that go through extensive adaptive changes are suggested to be more prone to slowly progressing degenerative processes. Mesulam put forward the plasticity failure hypothesis in order to explain limbic system vulnerability to pathologic tau protein phosphorylation, which is the core protein within the intraneuronally deposited neurofibrillary tangles (NFTs) in Alzheimer's disease (Mesulam, 1999). Briefly stated, as explained by this theory, limbic system is the most plastic structure in terms of acquisition and storage of recent memory traces, and in this respect primary sensory-motor cortices are the least plastic structures; hence the vulnerability of the former and the resilience of the latter structures to NFTs in AD. Likewise, it may be suggested that adapting to underwater conditions could pose more plastic load upon the visuo-spatial structures of the right hemisphere, rather than the linguistic structures of the left.

Sustained exposure to extreme environment can affect cognitive performance. Reed et al. (2001) found a significant decline of cognitive performance over a period of four months during Antarctic residence. Maruff et al. (2006) measured cognitive performance in healthy volunteers exposed to desert heat compared with unexposed individuals. Psychomotor speed, attentional and executive functions, but not performance accuracy, decreased as the expedition progressed.

We do not think that the ERP findings implicating a progressive deleterious effect and NTB findings suggesting an early gain and a late loss are contradictory. Although both are indices of cognitive functioning, their respective findings are not directly translatable into each other. Both have their advantages and limitations. Neuropsychological evaluation investigates the cognitive functions assuming a modularly and asymmetrically organized cognitive architecture and its findings are indirectly attributed to one or other dysfunctional cognitive module, which can sometimes be directly proven showing an imaging correlate. On the other hand, ERPs are bio-signals that are associated with direct on-line functional status of neural assemblies. In the present study, ERPs were sensitive to an ongoing subtle decline in attentional skills that NTB remains blind; on the other NTB revealed certain losses and gain in planning ability. Thus, we think that as shown in this study, the parallel use of both methods may reveal complementary findings overcoming the limitations inherent in each method.

5. Conclusions

SCUBA diving does not lead to major deleterious effects and clinical incidences when accidental cases of DCI is prevented, thus possible side effects of SCUBA diving in brain function are not widely considered as a matter of concern. Furthermore, the present NTB results suggest that acquisition of a complex skill such as diving might initially enhance planning abilities of individuals in order to deal with the extreme conditions posed by the underwater environment. However, these enhanced planning abilities seem to be lost overtime. Additionally, ROCFT-DRP and BDT results showed that divers with a longer diving history mildly lose their right hemispheric visual episodic memory and visuo-constructional skills.

We showed significant differences in amplitude and latency of several ERP waves between two groups of divers without DCI and non-divers. Other than the decline in a few NTB measures mentioned above, major behavioral deficits or complaints were not reported in those divers. The ERP results point to weaker and slower activity of certain neural assemblies of cerebral cortex engaged in stimulus discrimination, categorization, decision to act and response inhibition in occupational SCUBA divers. Although a prospective study with larger sample size would certainly provide more convincing evidence for investigating effects of diving on brain functioning, this study provides additional data to interrogate effects of long term diving exposure without DCI. As a concluding remark, we suggest inclusion of psycho-motor and cognitive performance tests into periodic check-up of professional divers. In the light of these assessments, more conservative procedures might be considered covering issues beyond presence or absence of DCI symptoms to screen and protect occupational divers from long-term effects of SCUBA diving.

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