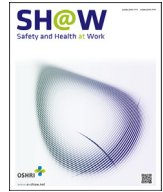




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Original article

Cardiac Autonomic Control and Neural Arousal as Indexes of Fatigue in Professional Bus Drivers

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ABSTRACT

Background: Bus driving is a mentally demanding activity that requires prolonged attention to ensure safety. The aim of the study was to assess mental fatigue caused by driving a public bus and to find a profile of workers at higher risk.

Methods: We evaluated changes of critical flicker fusion (CFF) (index of central arousal) and heart rate variability (HRV) (index of autonomic balance) in a 6-hour driving shift on a real route, in 31 professional bus drivers, and we tested the influence of personal factors such as sleep quality, BMI, and age. Paired t-test was used to test differences of CFF and HRV between both initial and final phase of driving, while multiple linear regression tested the influence of personal variables on the indexes of mental fatigue.

Results: Results showed that CFF significantly decreased after 6 hours of bus driving (41.91 Hz, sd 3.31 vs. 41.15 Hz, sd 3.15; $p = 0.041$), and heart rate significantly decreased in the final phase of driving, with respect to the initial phase (85 vs. 78 bpm, $p = 0.027$). Increasing age ($\beta = -0.729$, $p = 0.022$), risk of obstructive sleep apnea syndrome ($\beta = -0.530$, $p = 0.04$), and diurnal sleepiness ($\beta = -0.406$, $p = 0.017$) showed a significant effect on influencing mental fatigue.

Conclusion: Elderly drivers at higher risk of sleep disorders are more prone to mental fatigue, when exposed to driving activity. Monitoring indexes of central arousal and autonomic balance, coupled with the use of structured questionnaires can represent a useful strategy to detect profile of workers at higher risk of mental fatigue in such duty.

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1. Introduction

Urban public transport represents a highly important sector for the sustainable mobility of people. The assessment of the human factor applied to the transport sector needs constant research on how traffic road stressful stimuli can influence safety of drivers and passengers [1]. Bus driving is a working activity requiring a high level of attention and low level of energy expenditure [2]. Long working hours, monotonous activity, isolation and lack of interaction, non-equilibrated meals, and shift work could contribute to having detrimental effects on drivers' health [3]. Peculiar working hours, characterized by an early start in the morning and a late stop in the night, can lead to sleep deprivation and insomnia, that are relevant causes of driving accidents in bus drivers [4,5].

Moreover, bus drivers could be affected by sleep disorders, such as obstructive sleep apnea syndrome (OSAS), which can be associated with fatigue and sleepiness and road accidents [6]. Sleep disorders increase the risk of cardiovascular disorders, mental distress, and psychiatric illness [7–9] which can, in turn, influence alertness of bus drivers, increasing the risk of accidents and inducing fatigue.

Fatigue can be defined as a result of prolonged mental or physical exertion, affecting people's performance, and impairing their mental alertness, which leads to a higher risk of errors, incidents and accidents [10,11]. It is known that diurnal sleepiness and sleep disorders such as OSAS represent relevant factors able to lead to early mental fatigue, especially in monotonous tasks [12]. Mental fatigue can be intended as a failure to complete mental tasks that require self-motivation and internal cues in the absence

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of demonstrable cognitive failure or motor weakness [13]. Fatigue could have short-term effects such as impaired concentration, reduced hand–eye coordination, visual perception and vigilance, delayed reaction times, and cardiovascular alterations [10,14]. In the task of bus driving, some organizational factors such as long working hours, shift work, and starting work early in the morning can lead to sleep disturbance and sleepiness [12], which in turn significantly contributes to the onset of fatigue [15]. For these reasons, it is of crucial importance to investigate the onset of fatigue during such mentally demanding activity, to prevent accidents and injuries for drivers, passengers and road users.

As reported by previous studies, the onset of mental fatigue can be properly investigated by non-invasive methods, such as the analysis of the changes of the critical flicker fusion (CFF) frequency [16,17] and heart rate variability (HRV) [18,19].

The flicker fusion frequency has been used for psychological and behavioral evaluation [20]. Studies suggested that a decrease in CFF, as a consequence of simulated airplane flying activity or mental demanding tasks, represents an index of mental fatigue [21–23].

HRV analysis represents a useful method to assess cardiac autonomic response to environmental and organizational factor exposure [24,25]. Previous researches reported that cardiac autonomic balance changed as a consequence of fatigue, providing evidence on how the cardiovascular system can be affected by many stimuli from the environment [26,27].

To the best of our knowledge, no studies have investigated simultaneously CFF and HRV changes as objective indexes of mental fatigue due to driving a public bus. Several experimental studies found an association between alcohol intake and indexes of mental fatigue such as CFF [28], while patients affected by OSAS often reported fatigue symptoms and diurnal sleepiness [29]. Moreover, blood pressure has been reported as a predictor of fatigue in some working scenarios [30]. Nevertheless, no studies have simultaneously assessed personal variables as factors able to influence onset of fatigue, to find profile of workers at higher risk.

The aim of the present study was twofold: first, we aimed to evaluate the onset of fatigue in a sample of professional public bus drivers, by means of the analysis of objective detectable indexes of central arousal (CFF) and cardiac autonomic balance (HRV) in a real working scenario. Second, to find a profile of workers at higher risk of mental fatigue onset, we investigated the effect of personal factors such as age, sleep quality, risk of OSAS, alcohol intake, BMI, and blood pressure on the objective indexes of fatigue, focusing on those parameters easily detectable during periodic health check-up.

2. Materials and methods

2.1. Procedure and population

A cross-sectional study design was performed from October to December 2018. Thirty-two professional expert bus drivers (31 male and 1 female) belonging to an Italian public service bus company of over 2500 employees (participation rate 1.3%) were enrolled to participate in the study. Study participation was totally voluntary and actively promoted by the company supervisors who explained the study procedures and purposes, to get a continuous improvement of work organization. Each enrolled subject underwent a medical screening before the experimental test. Inclusion criteria were to be in the workforce of the company for at least 1 year as a bus driver, with a valid driving license. Participants must not suffer from any overt disease. All enrolled subjects were free of overt disease and before starting medical checks signed an informed consent to participate in the study.

Two weeks after the medical evaluation and questionnaire administration, the experimental phase began: drivers were

monitored during a real shift driving a bus along a suburban or extra-urban route, lasting about 6 hours. Each participant underwent two different driving routes, the first one in a suburban route and the second one in an extra-urban route. The two routes were performed in two different time slots: the first slot was in the early morning, with a start between 04:30 AM and 06:30 AM, and the second slot was in the afternoon, with a start between 01:00 PM and 03:00 PM. Each participant performed both the routes and both the time slots, except for one subject who did not performed the afternoon route, due to unpredictable change of work shift.

A flicker fusion frequency test evaluation was performed before starting driving activity and immediately after the end of the route. Moreover, a subsample of participants underwent a continuous ECG monitoring during driving activity, to assess HRV changes due to driving activity. The two driving tests were performed a week apart for each participant.

Data were safely stored and anonymized by providing a unique numeric code. The information collected was not to be shared with the company, except for anonymous aggregated data. The study adhered to the Helsinki declaration. Ethical review and approval were waived, due to its observational nature, in the absence of any additional procedure beyond mandatory risk assessment activity and mandatory health surveillance of the exposed workers, as laid down in the Italian Legislative Decree 81/2008, and in the absence of any involvement of therapeutic medication.

2.2. Medical evaluation

Each participant who agreed to participate in the study underwent a medical check, to evaluate whether inclusion and exclusion criteria were met, and collect personal and anthropometric variables. The following data such as gender, age, job seniority, company seniority, height, weight, body mass index (BMI), systolic and diastolic blood pressure (DBP), previous and actual diseases and medications, and ECG at rest were collected. All the participants met inclusion criteria, while none of them were excluded due to overt disease. Among 32 selected participants, 1 participant refused to participate in the experimental phase, after medical evaluation, leaving the sample of 31 participants who agreed to perform CFF and HRV assessment related to bus driving activity.

2.3. Questionnaires tools

Each participant was asked to answer a structured validated questionnaire, to assess the quality of sleep and sleep disorders, diurnal sleepiness, the risk of OSAS, and the risk of alcohol disorders. Questionnaires were administered by a well-trained occupational physician.

The quality of sleep was evaluated by the Pittsburgh Sleep Quality Index [31]. The score ranged from 0 (good sleep quality) to 21 (poor sleep quality). A score higher than 5 indicated a low sleep quality.

The risk of OSAS was assessed by the STOP-BANG Questionnaire [32], ranged from 0 (low risk of OSAS) to 8 (high risk of OSAS). Patients with a STOP-BANG score of 0 to 2 can be classified as low risk for moderate to severe OSAS, whereas those with a score of 5 to 8 can be classified as high risk for moderate to severe OSAS.

The diurnal sleepiness was assessed by Epworth Sleepiness Scale (ESS) questionnaire, ranging from 0 to 24. A score over 10 indicated excessive diurnal sleepiness [33].

The risk of alcohol disorder was assessed by the AUDIT-C questionnaire [34].

A score higher than 5 in men and 4 in women indicated a relevant risk of alcohol disorder.

2.4. Flicker fusion frequency test

To assess neural arousal, a flicker fusion frequency test was performed by a Flimmer Tubus (Schuhfried GmbH, Mödling, Austria). Each participant performed the test before (basal CFF) and after (final CFF) driving the bus on each of the two routes, resting in a sitting position for 15 minutes before the activity began and immediately after the end of the bus route.

The Flimmer Tubus providing a red flickering light stimulus was used to determine the CFF frequency. The participants were instructed to be in a sitting position in a quiet environment with a standardized comfort temperature of 24°C.

The test, lasting about 10 minutes, consisted in signaling, pressing a button, when a flicker light ranging from 7 Hz to 80 Hz, at a frequency progressively higher, was perceived as steady (fusion frequency – FF, Hz), and when the same light at frequency progressively lower was perceived as flickering (flicker frequency – VF, Hz). Each test consisted of almost 10 sets of data. The arithmetic mean of these data was the CFF. CFF was detected in increasing mode (the light from flickering becomes steady) and decreasing mode (the light from steady becomes flickering). In a training phase, each participant was instructed to watch the monitor of the instrument and was invited to determine the progressive switch from flicker light to stable light (ranging from 7 Hz to 80 Hz) and vice-versa. The training phase lasts about 3 minutes (about five training tests). The training phase automatically stopped when the standard error for the set of training test was lower than 1.0, ensuring a sufficient quality of the responses.

Each participant overall performed four CFF tests (two subsets of tests per each of the two routes). Only one participant did not perform the CFF test related to one route, leaving an overall sample of 122 CFF tests.

2.5. Heart rate variability recording

In a subsample of 18 drivers, a continuous ECG was performed during a work shift consisting of driving a bus in an actual scheduled route, for about 6 hours. The ECG tracks were recorded for the entire monitoring period using a three-channel (five-lead) digital Cardiette model HR one (Cardiette et medical devices SpA, Cavarano TN, Italy), with a sampling time of 250 Hz, equipped with the ECG Pilot and giOtto v.7.0.1.26 software (TBR, Gallarate, Italy).

Raw data were extracted by the software giOtto and analyzed with the Kubios HRV standard v. 3.4.3 software (Kubios Oy, Kuopio, Finland) [35], applying a medium intensity filter to eliminate artifacts. Heartbeat annotations were automatically assigned by the software and reviewed by a trained physician. Only normal sinus heartbeats were used for calculating the HRV parameters. One ECG trace with more than 5% of artifacts was found, so it was excluded from the analysis, leaving the final sample of 17 participants. The subgroup of 17 participants was comparable in terms of age, job seniority, BMI, and blood pressure with the overall study population (t -test $p > 0.05$).

HRV parameters were determined during driving activity over several intervals of 10 minutes: in the first 10 minutes of driving (initial phase), after 3 hours of driving (central phase), and after 6 hours of driving, at the end of the shift (final phase). The following HRV parameters in time domain and frequency domain were calculated: heart rate (HR, bpm), standard deviation of normal-to-normal (NN) intervals (SDNN, ms); root mean square of successive differences in adjacent NN intervals (RMSSD, ms); high-frequency (HF) power (ms²) in the range 0.15 – 0.40 Hz; LF/HF ratio.

HRV parameters were determined during driving activity over several intervals of 10 minutes: in the first 10 minutes of driving (initial phase), after 3 hours of driving (central phase), and after 6

hours of driving, at the end of the shift (final phase). The following HRV parameters in time domain and frequency domain were calculated: HR (bpm), SDNN (ms); RMSSD (ms); HF power (ms²) in the range 0.15 – 0.40 Hz; LF/HF ratio [25,36].

SDNN represents the total variability of HR, and it is influenced by both parasympathetic nervous system (PNS) and sympathetic nervous system activity. The RMSSD is mainly influenced by the PNS activity. HF power mainly reflects parasympathetic activity. LF/HF ratio represents a balance between the two branches of autonomic nervous system [25,36].

2.6. Statistical analysis

The normal distribution was checked by the Kolmogorov–Smirnov test and visual inspection of the distribution curves. All the HRV and CFF variables, except HF power fulfil the normality test, so a log-transformation was performed.

Mean and standard deviation of the parametric variables such as CFF, HRV, age, job seniority, BMI, systolic blood pressure (SBP) and DBP were calculated. Median and interquartile range were calculated for non-parametric variables Pittsburgh, ESS, STOP-BANG, and AUDIT-C scores.

We tested the differences between basal and final CFF parameters (VF and FF) by means of paired t -test. In the same way, we compared initial, central, and final HRV values by paired t -test. We also compared the final CFF and HRV values by time slot (morning vs. afternoon) and by the type of route (suburban vs. extra-urban) with t -test.

Correlations between personal variables and CFF and HRV parameters were calculated with the Pearson's correlation test or Spearman's correlation test, as appropriate. We considered the correlation weak in magnitude when r value ranged from 0.1 to 0.39, moderate with r between 0.4 and 0.69, strong with r between 0.7 and 0.89, and very strong with r between 0.9 and 1.0 [37].

Finally, CFF and HRV final parameters were individually predicted by linear regression modeling, as a function of personal parameters. CFF values (VF and FF) were predicted as a function of age, BMI, sleep quality (Pittsburgh score), diurnal sleepiness (ESS score), risk of OSAS (STOP-BANG score), risk of alcohol disorders (AUDIT-C score), and adjusted for the time of the test (morning or afternoon/evening) and the type of route (suburban or extra-urban). HRV final values were predicted by age, BMI, SBP, sleep quality, diurnal sleepiness, risk of OSAS and AUDIT-C, and adjusted for the time of the test. Beta coefficients were mutually adjusted for each predictor. The analyses were conducted using SPSS (v. 24, package for Windows, SPSS Inc., Chicago, IL, USA). The null hypothesis was rejected when associated with an α -error of 0.05.

3. Results

Characteristics of the study population are reported in Table 1. Mean BMI of 27.9 indicates an overall overweight of the study population [38], while mean SBP and DBP [39], sleep quality, risk of OSAS, diurnal sleepiness, and alcohol consumption were in the normal ranges.

The comparison between basal and final CFF values showed a statistically significant decrease of FF ($p = 0.041$). Mean decrease of -0.74 Hz was overall small by magnitude. On the other hand, final VF values did not show any significant change with respect to the basal values ($p = 0.13$) (Table 2). No significant differences were found when comparing CFF final values between morning and afternoon shift, and between suburban and extra-urban routes (Table 2).

When comparing initial and final HRV parameters, we found a significant decrease for mean HR in the final phase, with respect to

Table 1

Characteristics of the study population and scores of sleep quality, obstructive sleep apnea syndrome risk, diurnal sleepiness, and alcohol consumption

Parameters	Mean	sd	Optimal range
Age (years)	42.55	8.26	—
Job seniority (years)	14.44	8.29	—
Body mass index (Kg/m ²)	27.95	4.56	<25 Kg/m ²
Systolic blood pressure (mmHg)	127.97	13.25	<130 mmHg
Diastolic blood pressure (mmHg)	84.53	9.19	<85 mmHg
Median		IQR	
Pittsburgh	3	2–4	<5
STOP-BANG	1	1–3	<3
Epworth Sleepiness Scale	3	1.75–4	<10
AUDIT-C	1.5	0–3	<5 men; < 4 women

the initial phase ($p = 0.027$; mean variation -6.4 bpm) (Table 2). A significant decrease of mean HR was also found between initial and central values ($p = 0.0001$; mean variation -7.05 bpm) (Table 2). RMSSD varied with a change on the border of significance ($p = 0.090$), despite not significant by definition. No significant changes were detected for other HRV variables, both in the time domain and in the frequency domain. When comparing HRV parameters by time slot of the shift (morning vs. evening/afternoon), we do not found any significant differences for all the HRV parameters ($p > 0.05$).

Pearson's correlation test showed that VF variation has a weak significant inverse correlation with Pittsburgh score ($r = -0.389$, $p = 0.033$), while FF variation had a moderate inverse correlation with SBP ($r = -0.440$, $p = 0.013$). Age and job seniority were also moderately correlated ($r = 0.48$, $p < 0.005$). Moreover, we found a weak correlation between age and STOP-BANG ($\rho = 0.331$, $p = 0.01$) and between STOP-BANG and ESS ($\rho = 0.348$,

$p = 0.007$). No concerns of interrelation were detected for the predictors included in the regression models.

The results of the multiple linear regression predicting CFF final values as a function of personal variables and adjusted for the time of the test and the type of the route are reported in Table 3. ESS score showed a significant effect decreasing CFF fusion frequency (Beta = -0.406 , $p = 0.017$), while the predictors did not show any significant effect on the VF. The models accounted for 25% of the explained variance for FF, and for 18% of the explained variance for VF (Table 3).

When predicting HRV final values as a function of personal variables and adjusting for the time of the test (morning or afternoon/evening) we found that age and risk of OSAS significantly decrease SDNN (beta = -0.729 , $p = 0.022$, and beta = -0.530 , $p = 0.040$ for age and STOP-BANG score, respectively). Selected predictors explained 79% of the variance of SDNN (Table 4), while diurnal sleepiness was significantly positively associated with RMSSD, with 68% of variance explained by the model (Table 4).

4. Discussion

By an experimental cross-sectional study design, the present research aimed to evaluate the onset of mental fatigue assessing measurable parameters in a sample of professional bus drivers and investigating the influence of biological and personal variables.

Our results showed a significant change of CFF and HR due to bus driving activity. Moreover, several personal parameters such as age, diurnal sleepiness, and risk of OSAS showed a significant effect on CFF and HRV changes and, as a consequence, on the onset of fatigue.

Our results highlighted a significant decrease of CFF, but minor in magnitude and only for the FF parameter. This results are in line with previous studies, reporting a relevant decrease of CFF as a consequence of an exposure to stressful mental stimuli, such as flying an aircraft in a simulated scenario [21], or performing a mental task [40]. It is known that cognitive processes increases CFF

Table 2

Critical flicker fusion (basal and final) and HRV (initial, central, and final) parameters (mean, sd), and paired t-test results

CFF measures	Basal		Final		t-test basal versus final					
	Mean	sd	Mean	sd	t	p				
VF (Hz)	38.033	2.13	37.80	2.23	1.51	0.13				
FF (Hz)	41.91	3.31	41.15	3.15	2.08	0.041*				
Morning		Afternoon		t-test morning versus afternoon						
Final VF	37.948	2.1948	37.650	2.3027	0.518	0.606				
Final FF	41.610	3.1587	40.677	3.1344	1.158	0.252				
Suburban		Extra-urban		t-test morning versus afternoon						
Final VF	37.605	2.0881	38.300	1.9887	-1.044	0.302				
Final FF	40.516	2.7718	41.485	2.4765	-1.112	0.272				
HRV measures	Initial		Central		Final		t-test initial versus central phase		t-test initial versus final phase	
	Mean	sd	Mean	sd	Mean	sd	t	p	t	p
HR (bpm)	85.12	9.02	78.07	7.33	78.75	6.12	4.928	0.0001*	2.427	0.027*
SDNN (ms)	43.14	8.37	42.70	10.61	42.67	10.46	0.168	0.869	0.210	0.836
RMSSD (ms)	39.03	8.78	35.00	7.65	35.18	9.73	1.430	0.172	1.807	0.090
HF power (ms ²)	332.47	153.22	333.59	188.38	326.06	157.00	-0.020	0.984	0.135	0.894
LF/HF	4.00	2.65	4.36	2.63	4.06	2.55	-0.641	0.713	-0.145	0.886

* $p < 0.05$.

CFF: critical flicker fusion; HRV: heart rate variability; VF: flicker frequency; FF: fusion frequency; HR: heart rate; SDNN: standard deviation of normal-to-normal (NN) intervals; RMSSD: root mean square of successive differences in adjacent NN intervals; HF: high-frequency power in the range 0.15 – 0.40 Hz; LF/HF ratio.

Table 3
Multiple linear regression results predicting final critical flicker fusion (CFF) as a function of personal variables

	Final VF		Final FF	
	Adj beta	p	Adj beta	p
Age (years)	-0.174	0.221	-0.063	0.640
BMI (Kg/m ²)	0.296	0.065	0.216	0.158
Pittsburgh	0.199	0.207	-0.039	0.794
ESS	-0.112	0.516	-0.406	0.017*
STOP-BANG	0.061	0.720	-0.006	0.973
AUDIT-C	-0.257	0.113	-0.059	0.703
Time of test	-0.153	0.282	-0.138	0.310
Type of route	0.053	0.699	-0.076	0.565
R ²	0.182		0.249	

**p* < 0.05; Adj beta: adjusted beta coefficients.

threshold [41], therefore in this specific activity, the concomitant presence of monotonous phases alternated with interactive tasks could have contributed to prevent a higher drop of neural arousal. Our result in turn confirmed the hypothesis that driving activity affects neural arousal and the onset of fatigue can be detected by an objective evaluation tool such as the CFF test.

A significant decrease of HR was found in the central and in the final phase of driving activity. This relevant result suggests that the autonomic adjustment occurs already after the first 3 hours of shift. Decrease of HR is mainly caused by parasympathetic autonomic nervous activity [42], suggesting a predominance of parasympathetic autonomic balance, characterized by a drop of sympathetic autonomic activity [43]. The described autonomic balance seems not to be only explained as a function of circadian adjustments, since the comparison between HRV final values for the morning and afternoon/evening shift do not support this evidence.

As reported in previous articles, parasympathetic cardiac tone determine a decrease of HR, and a decrease of LF/HF ratio [36,44–46]. Our results showed that both HR and LF/HF ratio decreased, but only HR reached a statistically significant result. This aspect can be partially explained by the characteristics of such working activity, with multiple stressful stimuli from the environment that can lead to continuous adjustment of the autonomic nervous system. Such adjustments make HRV parameters depending on the moment of the sampling, while mean HR over a period of 10 minutes is more stable to rapid changes. Consequently, our results suggest that mean HR has a better reliability with respect to other HRV variables on monitoring the onset of fatigue in bus driving activity.

Table 4
Multiple linear regression results predicting HRV as a function of personal variables

Predictors	HR		SDNN		RMSSD		HF		LF/HF	
	adj beta	p	adj beta	p	adj beta	p	adj beta	p	adj beta	p
Age (years)	0.066	0.843	-0.729	0.022*	0.249	0.454	-0.291	0.574	-0.594	0.134
Job seniority (years)	-0.080	0.804	0.361	0.177	-0.377	0.254	0.082	0.868	0.676	0.087
Body mass index (Kg/m ²)	-0.317	0.435	-0.056	0.857	-0.269	0.498	0.062	0.919	-0.156	0.721
Systolic blood pressure (mmHg)	-0.109	0.688	0.450	0.063	0.253	0.357	0.105	0.801	0.205	0.497
Pittsburg	-0.880	0.064	-0.017	0.959	-0.531	0.219	-0.138	0.830	0.171	0.709
Epworth Sleepiness Scale	-0.069	0.813	0.351	0.154	0.664	0.048*	0.120	0.791	0.166	0.609
STOP-BANG	0.449	0.140	-0.530	0.040*	-0.228	0.418	-0.172	0.693	-0.471	0.156
AUDIT-C	0.527	0.086	0.112	0.605	-0.038	0.887	0.184	0.667	-0.047	0.877
Time of test	-0.618	0.090	-0.111	0.665	-0.208	0.522	-0.216	0.670	0.124	0.730
R ²	0.665		0.797		0.677		0.203		0.596	

**p* < 0.05.

HR: heart rate; SDNN: standard deviation of normal-to-normal (NN) intervals; RMSSD: root mean square of successive differences in adjacent NN intervals; HF: high-frequency power in the range 0.15 – 0.40 Hz; LF/HF ratio.

Moreover, the drop of HR already after 3 hours of driving suggests to avoid long driving for the entire shift, taking periodical restorative breaks along the shift. Thus, we can assert that long hours bus driving activity is able to determine a detectable onset of mental fatigue, as assessed by relevant changes of the cardiac autonomic control, with a predominance of parasympathetic tone with respect to the sympathetic activity. As reported in previous research, performing mental tasks showed a significant effect on HRV changes [47,48], suggesting a putative role of mental fatigue on influencing cardiac autonomic control of the heartbeat [49,50].

Several personal variables showed a significant effect on the indexes of fatigue. In detail, age and risk of OSAS showed a significant effect on decreasing SDNN, and diurnal sleepiness significantly increase RMSSD, suggesting an overall increase of sympathetic activity [25]. Moreover, diurnal sleepiness significantly affected CFF. As reported in a previous research, spectral HRV parameters correlated with sleepiness in a sample of young male participants exposed to partial sleep deprivation [51]. The relevant influence of sleep disorders on HRV and CFF supports the importance of the early detection of sleep disorders such as OSAS and excessive diurnal sleepiness in high responsibility activity such as professional bus drivers. The routine use of structured questionnaire to investigate sleep quality and risk of sleep disorders could be recommended in professional drivers, representing a useful tool to identify profiles of workers at higher risk of expire mental fatigue during driving activity. An ergonomic design of the shift schedule, and an individual counseling addressed to provide information on sleep hygiene during periodical health check-up, could also contribute to avoid sleep deprivation issues.

Taken together, these results support the hypothesis that a mentally demanding activity such as public bus driving is able to determine measurable changes of the parasympathetic tone and neural arousal. Moreover, those indexes of fatigue are significantly influenced by indexes of low sleep quality and risk of sleep disorders, assessed by structured validated questionnaires.

Some limitations affect the present study. First, our study design lacks a non-exposed control group. Nevertheless, the comparison with a non-exposed group is beyond the objective of the present study, further investigations are warranted to confirm the putative role of bus driving activity on causing changes of CFF and HRV. Second, the small sample size could also hide a relatively small association between the exposure to stressful stimuli and changes of measurable indexes of fatigue.

Third, we recruited a small rate of participants, with respect to the total workforce of the company, so the generalizability of the results could be affected by this limitation. Fourth, the wide

confidence interval of the measurements carried out could have contributed to hide small changes of the investigated parameters.

Finally, we do not assess energy expenditure during the task. Driving activity requires a level of energy expenditure between 2.0 and 2.8 metabolic equivalents of task, corresponding to a light-intensity activity [52]. For this reason, we do not suppose that the driving task could significantly determine an onset of physical fatigue.

Despite such limitations, our study provides new insight on the knowledge related to the onset of fatigue in professional bus drivers. Our experimental in-field study, which supported the hypothesis that a task characterized by high demand in terms of attention and concentration, determines objective changes of CFF and HRV as indexes of mental fatigue. Moreover, to identify workers at higher risk of fatigue consequences, our results highlight the importance of screening bus drivers for sleep disorders during periodical health check-up.

The results of the present study could be useful for working duties characterized by similar working conditions (e.g., train engineer, pilot vessels, quay crane operators, airline pilot, air traffic controller, and so on). Thus, the beneficial effects of properly managing the issue of mental fatigue relate to the well-being of workers, the safety of users and the economics of organizations.

To implement health surveillance programs for this job task, the use of objective measurement tools, coupled with structured questionnaires can represent a useful strategy to detect workers at higher risk for early onset of fatigue, preventing them from dangerous errors during driving, especially for aging workers.

Further investigation on the fatigue onset of bus drivers and personal and behavioral factors able to affect workers' well-being are warranted, to implement health monitoring and improving strategies aimed to prevent injuries and accidents of professional bus drivers, passengers, and road users.

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Conflicts of interest

The authors declare no conflict of interest.

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