



## Effects of a cool classroom microclimate on cardiac autonomic control and cognitive performances in undergraduate students



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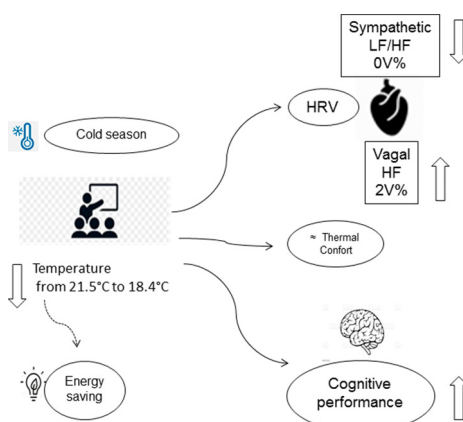
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### HIGHLIGHTS

- Analyses were carried out in NEUTRAL (21.5 ± 0.8 °C) and COOL (18.4 ± 0.4 °C) trials.
- Spectral and symbolic analyses provided indices of cardiac autonomic modulation.
- Enhanced vagal and reduced sympathetic cardiac modulation were observed during COOL.
- Cognitive performance was superior during COOL compared to NEUTRAL trial.
- Energy saving might be obtained during learning activities in the cold season.

### GRAPHICAL ABSTRACT



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### ABSTRACT

An inverted U-shape relationship between cognitive performance and indoor temperature with best performance peaking at 21.6 °C was previously described. Little is known on classroom temperature reduction effects on cognitive performances and cardiac autonomic profile, during the cold season.

Fifteen students underwent electrocardiogram recording during a lecture in two days in December when classroom temperatures were set as neutral (NEUTRAL, 20–22 °C) and cool (COOL, 16–18 °C). Cognitive performance (memory, verbal ability, reasoning, overall cognitive C-score) was assessed by Cambridge Brain Science cognitive evaluation tool. Cardiac autonomic control was evaluated via the analysis of spontaneous fluctuations of heart period, as the

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temporal distance between two successive R-wave peaks (RR). Spectral analysis provided the power in the high frequency (HF, 0.15–0.40 Hz) and low frequency (LF, 0.04–0.15 Hz) bands of RR variability. Sympatho-vagal interaction was assessed by LF to HF ratio (LF/HF). Symbolic analysis provided the fraction of RR patterns composed by three heart periods with no variation (0 V%) and two variations (2 V%), taken as markers of cardiac sympathetic and vagal modulations, respectively. The students' thermal comfort was assessed during NEUTRAL and COOL trials.

Classroom temperatures were  $21.5 \pm 0.8$  °C and  $18.4 \pm 0.4$  °C during NEUTRAL and COOL. Memory, verbal ability, C-Score were greater during COOL ( $13.01 \pm 3.43$ ,  $12.32 \pm 2.58$ ,  $14.29 \pm 2.90$ ) compared to NEUTRAL ( $9.98 \pm 2.26$ ,  $p = 0.002$ ;  $8.57 \pm 1.07$ ,  $p = 0.001$  and  $10.35 \pm 3.20$ ,  $p = 0.001$ ). LF/HF ( $2.4 \pm 1.7$ ) and 0 V% ( $23.2 \pm 11.1\%$ ) were lower during COOL compared to NEUTRAL ( $3.7 \pm 2.8$ ,  $p = 0.042$ ;  $28.1 \pm 12.2\%$ ,  $p = 0.031$ ). During COOL, 2 V% was greater ( $30.5 \pm 10.9\%$ ) compared to NEUTRAL ( $26.2 \pm 11.3$ ,  $p = 0.047$ ). The students' thermal comfort was slightly reduced during COOL compared to NEUTRAL trial.

During cold season, a better cognitive performance was obtained in a cooler indoor setting enabling therefore energy saving too.

## 1. Introduction

Uncomfortable indoor microclimate conditions may affect subjective thermal comfort, working and learning performance (Al Horr et al., 2016; Barbic et al., 2014a; Fang et al., 2004; Lorsch and Abdou, 1994a, 1994b; Vimalanathan and Ramesh Babu, 2014; Wang et al., 2017). The role of the microclimate parameters on cognitive performance has been widely studied (Abbasi et al., 2019; Chang and Kajackaite, 2019; Seppanen et al., 2003; Seppanen et al., 2006b) both during working (Griffiths and Boyce, 1971; Jensen et al., 2009; Kosonen and Tan, 2004; Pilcher et al., 2002) and learning (Haverinen-Shaughnessy and Shaughnessy, 2015; Kimura et al., 2020; Temprano et al., 2020) activities in order to optimize human performances in different cognitive tasks (Hancock and Vasmatazidis, 2003). Findings suggest an inverted U-shape relationship between cognitive performance and indoor air temperature within the range of observation 20–32 °C, with the highest performance peaking at 21.6 °C (Pilcher et al., 2002; Seppanen et al., 2006b).

In a recent systematic review of the literature, Brink and colleagues (Brink et al., 2020) underlined the increasing interest to identify the optimal microclimate conditions during learning activities. Indeed, better indoor environment characteristics proved to positively affect both the quality of learning and students' short-term academic results (Brink et al., 2020; Haverinen-Shaughnessy and Shaughnessy, 2015; Temprano et al., 2020), whereas thermal discomfort played a role in reducing cognitive performance (Brink et al., 2020). However, not all thermal discomfort sensations led to a decrease of cognitive performance (Siqueira et al., 2017) and it was shown that the final effect was most likely task dependent (Pilcher et al., 2002). Thus, the relationship between indoor microclimate and different areas of cognitive performance remains poorly understood (Brink et al., 2020).

The cardiovascular autonomic control plays a key role in thermoregulatory function (Castellani and Young, 2016; Greaney et al., 2016; Sawasaki et al., 2001). The acute physiological responses to cold exposure include vasoconstriction and shivering thermogenesis that involve the autonomic nervous system and, specifically, cardiovascular autonomic functioning (Castellani and Young, 2016; Greaney et al., 2016; Sawasaki et al., 2001). This latter is also crucial in maintaining attention level (Burov and Tsarik, 2012; Liu et al., 2008; Luque-Casado et al., 2013; Pagani et al., 1991). For example, during physical and mental activities required by the working tasks in a steel company, the spectral indices of cardiac sympathetic modulation were found to increase during the day while they decreased during sleep (Furlan et al., 2000a). Recent studies evaluating the relationships between cognitive function and cardiac autonomic control, suggested that the decrease of heart rate variability (Thomas and Viljoen, 2019) and the increase of cardiac sympathetic modulation indexes (Forte et al., 2019) could be associated with a worse performance compared to what was observed in the presence of cardiac vagal predominance. However, the link between autonomic nervous system functioning and cognitive performance remains elusive and poorly described.

Interactions between cardiac autonomic control, microclimate parameters and cognitive performance are complex (Barbic et al., 2019; Giuliano et al., 2017; Solhjoo et al., 2019; Thomas and Viljoen, 2019). Indeed, cognitive performance requires a dynamic interplay between sympathetic and

parasympathetic activity (Abbasi et al., 2019; Giuliano et al., 2017; Kimura et al., 2020) also depending on autonomic changes involved in the thermoregulatory functioning. The latter is strictly related to the outdoor microclimate, particularly in response to cold exposure (Castellani and Young, 2016).

In a recent study on undergraduate students during spring (Barbic et al., 2019), we observed that an increase in the classroom temperature of about 4 °C compared to the neutral condition (22 °C) was associated with a reduced global cognitive performance in the presence of a cardiac autonomic control shift towards a higher sympathetic predominance and a significant thermal discomfort (Barbic et al., 2019). In a previous pilot study by our group (Barbic et al., 2020), we assessed the effects of a reduction of about 4 °C on cognitive performance and on the indexes of cardiac autonomic modulation by symbolic analysis (Guzzetti et al., 2005; Porta et al., 2001) of heart rate variability. However, in this preliminary study the experiments were performed on two days characterized by a significant difference in out-door temperature (different period of the year, spring, and winter). In addition, the students' thermal comfort and the role of clothing were not considered. In the current study we firstly evaluated the effects of a reduction of classroom temperatures on cognitive performance and secondly the associated changes of cardiac autonomic control in a class of undergraduate students. Given that cognitive performance seemed to peak at about 21.6 °C (Pilcher et al., 2002; Seppanen et al., 2006b), the neutral classroom temperature was set at 20–22 °C. In addition, because the outdoor temperature may play a confounding role in the cardiac autonomic response (Castellani and Young, 2016), all recordings were performed in the winter thus reducing outdoor temperature variability.

## 2. Material and methods

The current investigation was approved by the Local Ethics Committee (#2153) and a written informed consent was signed by all the participants in the study.

### 2.1. Sample population

A group of 15 healthy students attending the Humanitas University School of Physiotherapy, (8 M, age  $20 \pm 2$  yrs., BMI  $23 \pm 2$  Kg/m<sup>2</sup>) were studied on two different days in the same classroom of the University Campus. It must be highlighted that 20 subjects were originally enrolled in the study; however, 5 of them had to be excluded from the final analysis. In detail, 2 subjects were absent from one of the trials (the Cool trial) because of the flu despite their participation in the neutral temperature trial. In 3 of those 5 students, the quality of the continuous ECG recordings during the two-hour lecture, in one of the two trials, was insufficient for adequate frequency domain and symbolic analyses. Therefore, the study ultimately included a total of 15 participants.

The day before the first trial, all students were preliminarily trained to use the Cambridge Brain Science (CBS) cognitive evaluation tool. This is the method that was subsequently used to assess their cognitive performance at the end of the two lectures. Such a training procedure was introduced to normalize for a potential “learning effect” bias. In addition, on the day of the trial students were kept unaware of the temperature set, to

**Table 1**  
Demographics and habits of the 15 students.

Variables	
Age (years)	20 ± 2
Gender (M/F)	8/7
BMI (kg/m <sup>2</sup> )	22.7 ± 2.3
Caffeine intake (%)	80
Cups/day	2 ± 1
Nicotine intake (%)	20
Cigarettes/day	6 ± 1
Alcohol intake (%)	53
Alcohol unit/week	3 ± 3
Prescription drug intake (%)	20
Physical activity (%)	67
Competitive athletes (%)	50
Allergies (%)	20
Sleep (hours/day)	7 ± 1

BMI indicates Body Mass Index. Alcohol unit equals 10 ml or 8 g of pure alcohol. Values are expressed as mean ± SD.

prevent changes in their habitual clothing. The students were asked to refrain from smoking and caffeine intake for at least 4 h before the beginning of the lecture. The demographic features, smoking, coffee and alcohol intake, and physical activity habits of the study participants are reported in Table 1. Sleep duration on the night before the NEUTRAL and COOL trials was similar ( $7.0 \pm 1$  and  $6.4 \pm 1$  h, respectively). All students were healthy, and none was on any medications during the study protocol.

## 2.2. Experimental protocol

The study protocol was performed during two lectures, on two different days where the classroom temperatures were set by the automatic air conditioning system to 20–22 °C on December 3th (NEUTRAL), and 16–18 °C on December 15th (COOL). The NEUTRAL trial was considered as a reference condition of the present study based on previous data reporting that the cognitive performance is likely to peak at 21.6° (Seppanen et al., 2006b). In Milan, the month of December is during the cold season, 154–155).

The experimental protocol is shown in Fig. 1. At T0, in a separate area (Instrumentation room), all students underwent instrumentation with individual placement of a portable device (MR&D Pulse, Italy) on the left side of the anterior thorax, for the continuous recording of a single lead electrocardiogram (ECG). During the instrumentation phase, the students were in a standing position to allow an easy placement of the ECG leads. Students were kept outside the classroom until the beginning of the lecture. About 1 h after instrumentation, an expert technician verified the adequate

functioning of all the devices (T1) and thereafter, the students entered the classroom, and the lecture began. One hour after the beginning of the lecture (T2), all the students filled out an ad hoc questionnaire to assess their thermal comfort during the lecture. At the end of the lecture, just before leaving the classroom (T3), each student underwent the CBS cognitive evaluation to assess their cognitive performance. After the registration of the CBS test results, ECG recordings were stopped, and the students were free to leave the classroom (T4). The single lead ECG was recorded concomitantly in all students from T0 to T4 and the signals were sent to a server for off-line analysis every 5 min by a telemetry system (HP Mobile H&S). The lecture, performed on two different days had the same duration (110 min) and topic, and was conducted by the same teacher to standardize the students' mental pre-load as much as possible, before the CBS.

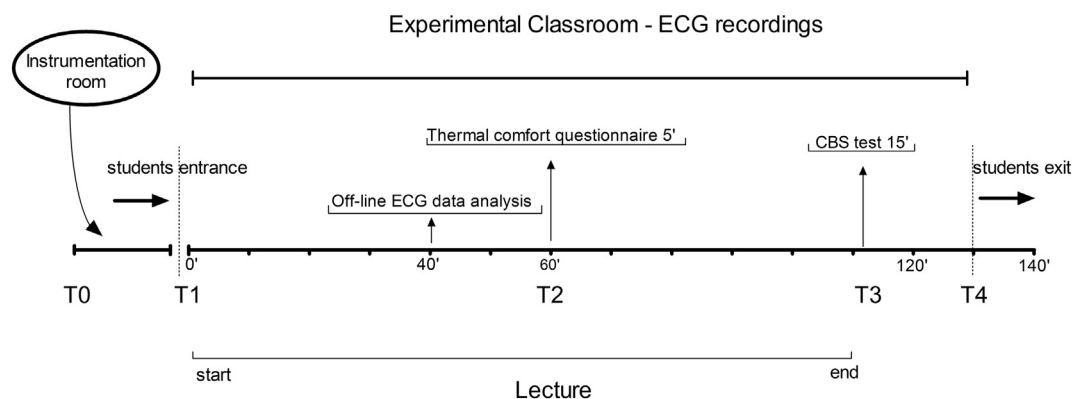
## 2.3. Microclimate parameters

The day before the beginning of the study, battery supplied sensors were placed in the Experimental Classroom to assess the temperature (°C), CO<sub>2</sub> (ppm) and relative humidity (%). The sensors were placed far away from windows and doors and were set to record the microclimate parameters every 30 s. Microclimate data were sent to a server by telemetry every 5 min. Devices used for measuring, recording, storing, and analysing the indoor microclimate parameters were developed and built by researchers (L.C. and M.S.) of the Politecnico of Milan, Italy.

The environmental outdoor parameters (temperature and relative humidity) were obtained by ad hoc sensors that were placed near the University facilities and were provided by the “Osservatorio Meteorologico Lombardo” (<http://www.centrometeolombardo.com>).

## 2.4. Cognitive performance

The Cambridge Brain Science (CBS) cognitive evaluation tool developed by Cambridge University (<https://www.cambridgebrainsciences.com/science/tests>) was used to assess the students' cognitive performance. The CBS tool evaluates three independent cognitive domains that are *short-term memory*, *verbal ability*, and *reasoning*. *Short-term memory* explores the ability to actively hold information in the brain while working on it. *Verbal ability* corresponds to the capability to produce and comprehend information with specific meaning. *Reasoning* is defined as the ability to manage information according to logical rules. The entire cognitive test battery lasted about 15 min. The *C-Score* is a synthetic index of cognitive performance, considering the three different cognitive domains previously assessed. The CBS cognitive evaluation does not provide a score range of normality. Scores are compared to a community database from tens of thousands of people. This database is a rough representation of the performance of the



**Fig. 1.** Experimental protocol. Main feature of the experimental protocol during the NEUTRAL and COOL trials (see test for details). CBS indicates Cambridge Brain Sciences cognitive test. ECG recordings started at T1 and ended at T4. Lectures started at T1 and ended after 110 min. At T2, during a short break, the students completed the Questionnaire on Thermal Comfort. The CBS test was performed at T3. Students' classroom entrance and exit were at T1 and T4, respectively. The “Instrumentation room” is a facility within the Humanitas University campus where we could place ECG electrodes on the thoracic skin of our students before the recording session. Such a room is close to the Experimental Classroom and its indoor microclimate is characterized by standard temperature and humidity set by the automatic air conditioning system.

general population. Currently, it is not possible to compare the individual scores with people of the same age.

## 2.5. Thermal comfort

The “Questionnaire for the Thermal Comfort Survey” modified by Wang Y (ASHRAE, 2010; Mohlenkamp et al., 2019; Wang et al., 2017) was used to assess the actual thermal comfort of the students in the middle of the lecture during NEUTRAL and COOL trials. The Questionnaire provided seven levels of “Thermal Comfort”. Participants were asked to rate their overall comfort as “1. Very comfortable”, “2. Moderately comfortable”, “3. Slightly comfortable”, “4. Neutral”, “5. Slightly uncomfortable”, “6. Moderately uncomfortable” and “7. Very uncomfortable”. A condition of “Thermal Comfort” was considered if the student's answer was either “neutral” or “higher degree” of comfort. The questionnaire also provided five levels of Thermal Preference Vote for air temperature, humidity, and air speed. The answers of “no change”, “a bit cooler”, “a bit warmer”, “a bit humid” and “a bit drier”, were considered as a sufficient degree of comfort. Finally, the students had to provide information about the type of clothing they were wearing. The individual clothing insulation was determined and expressed in *clo* unit according to the ASHRAE55-2012 standard, during the Neutral and Cool trials (ANSI/ASHRAE, 2012). The number of hours slept by the students the night before each trial was also quantified. The time needed to complete the questionnaire was about 5 min (Fig. 1).

## 2.6. Cardiac autonomic profile

Students' cardiac autonomic profile was assessed by spectral and symbolic analysis of heart period variability, starting 40 min after the beginning of the lecture (Fig. 1), in the sitting position. This time was chosen to allow for adequate students' acclimatization to the classroom temperature and adaptation to the sitting position in order to abolish any residual effect induced by the previous standing position during instrumentation.

The ECG recordings were sampled at 128 Hz and the ECG quantization level was 3.26  $\mu$ Volts. The heart period was automatically calculated on a beat-by-beat basis as the temporal distance between two consecutive R-wave peaks (RR interval) by Heart-Scope2© software. The R-wave detections were performed automatically off-line and possible misidentifications were manually corrected. To remove the presence of ectopic beats on RR interval series, a linear interpolation was applied. Only isolated ectopic beats were edited. A maximum of 5% of corrections was allowed over each frame of analysis. If the selected segment did not satisfy this condition, a new segment selection was carried out.

Series of 300 beats extracted from the continuous ECG recording were selected and analyzed, (Task-Force, 1996). The hypothesis of restricted weak stationarity (i.e. stationarity of the mean and variance) of the series was tested according to Magagnin et al. (Furlan et al., 2001; Magagnin et al., 2011). A linear de-trend was performed on RR series before analysis.

Respiration rate was obtained indirectly from ECG by the analysis of the beat-to-beat variations of the QRS amplitude (Moody, 1985; Porta et al., 1998).

## 2.7. Spectral analysis of heart rate variability

A full description of the parametric method for the assessment of the power spectral density based on autoregressive (AR) model is described elsewhere (Pagani et al., 1986; Task-Force, 1996). Briefly, the AR power spectral density was estimated over stationary sequences of 300 consecutive RR measures. Power spectral density was decomposed into components relevant to a real pole or a pair of complex and conjugate poles (Baselli et al., 1997). Each spectral component was labelled as low frequency (LF band, from 0.04 to 0.15 Hz) or high frequency (HF band, from 0.15 to 0.5 Hz) component whether the phase of the real pole or pair of complex and conjugate poles was within the LF or HF bands, respectively. The area under the spectral component, estimated via the residue theorem (Baselli et al., 1997), represents the variance associated to each

spectral component. It was expressed in  $\text{ms}^2$  and labelled as  $\text{LF}_{\text{RR}}$  and  $\text{HF}_{\text{RR}}$  whether it was relevant to LF and HF bands, respectively. Normalized power was obtained by dividing the  $\text{LF}_{\text{RR}}$  and  $\text{HF}_{\text{RR}}$  powers, by total variance diminished by the power of the very low frequency band (i.e., below 0.04 Hz) and multiplying by 100. Normalization procedure limited the dependence of  $\text{LF}_{\text{RR}}$  and  $\text{HF}_{\text{RR}}$  markers on the mean RR interval and its total variance individual differences.

As previously reported by several studies, the  $\text{LF}_{\text{RR}}$  in normalized units (n.u.) and LF/HF ratio increase in conditions characterized by an enhancement of the cardiac sympathetic activity such as during the up-right position (Furlan et al., 2000b), mental arithmetic tasks (Pagani et al., 1989), light physical exercise (Rimoldi et al., 1992), hypertension (Furlan et al., 1991), heart failure (Guzzetti et al., 2001) and others. Moreover, these indices are blunted or abolished during acute (Cogliati et al., 2004) or chronic beta blockade (Pagani et al., 1986), ganglionic blockade (Diedrich et al., 2003) or in patients with Parkinson's disease and orthostatic hypotension (Barbic et al., 2007), and in Pure Autonomic Failure (Furlan et al., 1995), a rare condition characterized by the neurodegeneration of the post-ganglionic sympathetic neurons innervating the sinoatrial node. As to the  $\text{HF}_{\text{RR}}$  component of RR variability, it may reflect cardiac vagal modulatory activity since it decreases during the head-up tilt position, a stimulus which decreases cardio-vagal modulation (Furlan et al., 2000b), or after muscarinic blockade with atropine (Pomeranz et al., 1985), and it is also abolished during complete ganglionic blockade (Diedrich et al., 2003). The LF/HF is a recognized index of the sympathetic-vagal interactions to the sinoatrial node (Barbic et al., 2014b; Furlan et al., 2000a; Furlan et al., 2000b; Furlan et al., 2001).

## 2.8. Symbolic analysis of heart rate variability

Symbolic analysis provides an alternative tool for decomposing heart rate variability series based on classification of patterns identified in the RR series (Porta et al., 2001) and has been profitably used to assess the cardiac autonomic profile in physiology and pathology (Porta et al., 2007; Porta et al., 2015a; Zamuner et al., 2019). Briefly, given the same RR sequence selected for spectral analysis, a uniform quantization procedure over 6 bins was applied to transform the RR series into a sequence of integers ranging from 0 to 5. This transformation converted the RR series in a sequence of symbols according to the framework of symbolic analysis (Porta et al., 2001; Porta et al., 2015a). Subsequently, from the integer sequence we built patterns of 3 consecutive integers. All patterns were grouped into 3 families (Guzzetti et al., 2005): patterns with no variation, termed 0 V, featuring 3 equal symbols; patterns with 1 variation, denoted 1 V, featuring two adjacent equal symbols while the remaining one was different; patterns with 2 variations, termed 2 V, featuring adjacent symbols that were different regardless of their position within the pattern. Results were expressed as percentage computed by dividing the number of the patterns belonging to the same family (i.e., 0 V, 1 V or 2 V), multiplied by 100, by the number of total patterns. These percentages were denoted with 0 V%, 1 V% and 2 V%.

The 0 V% is an index of cardiac sympathetic modulation since it increases during 80° head-up tilt (Porta et al., 2007), hand-grip (Guzzetti et al., 2005), nitroprusside intravenous infusion and total muscarinic blockade by high dosage administration of atropine (Guzzetti et al., 2005). Conversely, the 2 V% index, as a sum of 2 like (2LV) and 2 unlike (2UV) variations, is considered a marker of cardiac parasympathetic modulation because it increases after intravenous administration of phenylephrine which reflexively enhances the cardiac vagal drive (Guzzetti et al., 2005). The same index decreased after atropine administration, tilt manoeuvre (Porta et al., 2007) and hand-grip (Guzzetti et al., 2005).

## 2.9. Statistical analysis

The size of the group was decided based on Barbic et al. (2019). Since the difference in both cognitive performances and spectral profiles were significant after increasing temperature in 15 subjects, we took this as a sample size for the present work that separately considered the effect of a decrease in temperature.

Normality of distribution was checked using the Shapiro-Wilk test. The paired *t*-test was utilized to compare data of cognitive performance and indices of cardiac autonomic control between the NEUTRAL and COOL trials when the normality hypothesis was fulfilled. Otherwise, a Wilcoxon signed-rank test was applied. A Chi squared test was performed to compare the students' thermal comfort level during the two microclimate conditions.

Statistical analysis was carried out using a commercial statistical program (Sigmaplot, v.14.0, Systat Software, Inc., Chicago, IL, USA). Data are expressed as mean ± standard deviation (SD). A value of *p* < 0.05 was always considered significant.

### 2.10. Ancillary estimation of potential “energy saving”

In the present study we also reported the estimated energy consumption for the month of December, to maintain the indoor temperature of the teaching building at the set point corresponding to the Neutral trial (i.e., 21.5 ± 0.8 °C) and at the set point corresponding to the Cool trial (i.e., 18.4 ± 0.4 °C). The energy consumption data were provided by the Humanitas University Energy Management Office.

## 3. Results

The mean values of outdoor temperature and relative humidity on the two trials are reported in Table 2a. The actual microclimate parameters recorded in the classrooms are reported in Table 2b. Notice that the actual classroom temperature during the NEUTRAL trial (21.5 ± 0.8 °C) was in accordance with the value of the temperature set by the automatic air conditioning system (temperature set: 20–22 °C). Conversely, the temperature measured during the COOL trial (18.4 ± 0.4 °C) corresponded to the upper limit of the set temperature range (temperature set: 16–18 °C). The CO<sub>2</sub> values were <600 ppm during the two different trials, indicating efficient classroom ventilation during both lectures. The relative humidity was lower during COOL compared to the NEUTRAL trial, as it was technically impossible to maintain that parameter unchanged. The air velocity was lower than 0,1 m/s. The indoor microclimate parameters were stable during the trials.

The estimated thermal energy consumption during NEUTRAL and COOL trials, is shown in Table 2e. Please notice the potential energy saving evaluation when the classroom temperature was kept according to the Cool trial. The effects of lighting and cloudiness as well as the potential effect of different sun exposures were likely negligible in this experimental setting because the windows of the classroom were blacked-out to optimize visualization of the slides projected during the lectures.

### 3.1. Thermal comfort assessment

The percentage of students reporting “Thermal Comfort”, as defined in the Methods section, was lower during the COOL (26,7%) compared to the NEUTRAL (80%; *p* < 0.05) trials. Thermal Comfort and Thermal Predicted Votes are reported in Table 2c and d. Based on the Thermal Predicted Vote a sufficient degree of comfort for humidity was reported by 100% of the students, both during the Neutral and Cool trial. A sufficient degree of comfort for temperature was reported by 100% of the students during the Neutral trial and by 73% during the Cool trial (Table 2d). None of the students complained of discomfort due to air movement.

The question “What is your clothing now?” included in the questionnaire for the thermal comfort survey, provided information for evaluating the clothing insulation power. The latter was 1.12 ± 0.07 *clo* during both NEUTRAL and COOL trials.

### 3.2. Cognitive performance

The mean values of the cognitive performance scores are reported in Table 3 and the individual cognitive scores of each participant are shown in Fig. 2. During the COOL trial *short-term memory*, *verbal comprehension* and *C-score* were significantly greater compared to the NEUTRAL one.

**Table 2**

Microclimate parameters, thermal comfort perception, thermal predicted vote, and energy expenditure estimation during Neutral and Cool trials.

a. Outdoor microclimate.		
Outdoor parameters	Neutral	Cool
Measured temperature (°C)	8.9 ± 0.3	3.3 ± 0.6
Humidity (%)	81.2 ± 1.0	81.4 ± 1.7
b. Indoor classroom microclimate.		
Indoor parameters	Neutral	Cool
Temperature (°C)	21.6 ± 0.7	18.4 ± 0.4
CO <sub>2</sub> (ppm)	547 ± 95	562 ± 36
Humidity (%)	40.2 ± 0.8	29.0 ± 0.7
c. Indoor thermal comfort perception.		
Thermal comfort perception	Neutral	Cool
Very comfortable, N	2	0
Moderately comfortable, N	3	2
Slightly comfortable, N	4	2
Neutral, N	3	0
Slightly uncomfortable, N	3	7
Moderately uncomfortable, N	0	4
Very uncomfortable, N	0	0
<sup>a</sup> Thermal comfort, N (%)	12 (80)	4 (27)*
d. Indoor thermal predicted vote		
Temperature	Neutral	Cool
Much cooler, N	0	0
A bit cooler, N	1	0
No change, N	7	1
A bit warmer, N	7	10
Much warmer, N	0	4
Humidity	Neutral	Cool
Much drier, N	0	0
A bit drier, N	0	2
No change, N	13	12
A bit more humid, N	2	1
Much more humid, N	0	0
e. Energy expenditure estimation (December) <sup>b</sup> .		
	Neutral	Cool
Power (MWh)	6.8	5-5

Values are expressed as mean ± SD. N, indicates the number of subjects.

<sup>a</sup> Thermal comfort corresponds to the sum of answers: very, moderately, slightly comfortable, and neutral. \* *p* = 0.003.

<sup>b</sup> Energy expenditure estimation was computed as described in the methods 2.10 paragraph. MWh indicates Megawatts hour.

### 3.3. Cardiac autonomic profile

The indices of cardiac autonomic control, as assessed by both power spectral and symbolic analyses of heart rate variability (Fig. 1), are reported in Table 4. In Fig. 3, the single values of RR variance, LF/HF, 0 V%, 2 V% obtained in the two trials are shown.

During the COOL trial the symbolic index of cardiac sympathetic modulation (0 V%) was lower while the symbolic marker of cardiac vagal modulation (2 V%) was greater compared to the NEUTRAL one.

**Table 3**

Cognitive performance of the 15 students as assessed by the Cambridge Brain Science Cognitive Evaluation tool during the neutral and cool trials.

Cognitive test domains	Neutral	Cool	<i>p</i>
Short-term memory	9.98 ± 2.26	13.01 ± 3.43	0.002
Verbal ability	8.57 ± 1.07	12.32 ± 2.58	0.001
Reasoning	15.18 ± 2.39	14.97 ± 2.67	0.791
C-score	11.09 ± 1.59	14.29 ± 2.90	0.001

Values are expressed as mean ± SD.

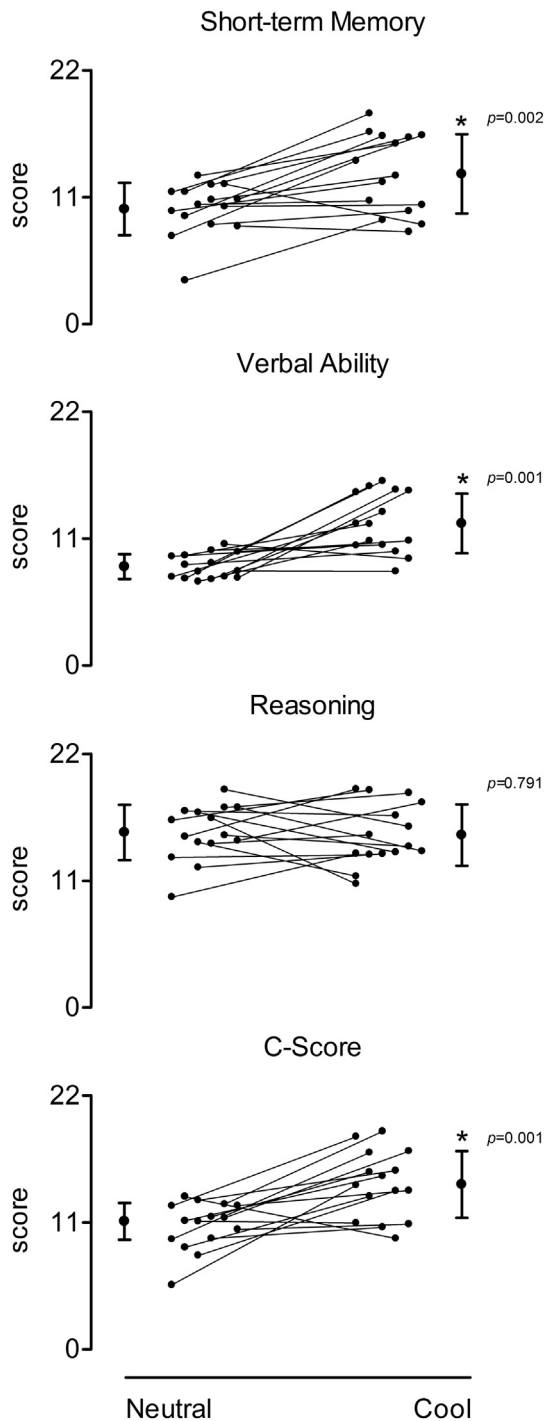


Fig. 2. Students' cognitive performance individual scores and their mean values with standard deviation during the NEUTRAL and COOL trials. Notice that during COOL, short-term memory, verbal ability and overall cognitive C-Score were significantly greater compared to the NEUTRAL trial.

The spectral index of cardiac sympatho-vagal modulation was lower on the COOL compared to the NEUTRAL trial. HR values and HR variance were similar in the two different trials.

#### 4. Discussion

The main results of the current study were as follows: 1) the reduction of the classroom temperature of about 3 °C compared to the reference condition, i.e., NEUTRAL trial, was associated with an increase in the students'

Table 4

Heart rate, spectral and symbolic analyses indices of R-R interval variability during neutral and cool trials.

Variables	Neutral	Cool	p
HR (bpm)	74.8 ± 8.4	73.4 ± 9.1	0.335
μRR (ms)	813.1 ± 108.8	829.1 ± 104.8	0.360
σ <sup>2</sup> RR (ms <sup>2</sup> )	5063.9 ± 2380.2	4768.5 ± 1264.9	0.509
LF <sub>RR</sub> (ms <sup>2</sup> )	1723.1 ± 1669.6	1551.0 ± 1092.7	0.666
LF <sub>RR</sub> (nu)	62.4 ± 23.3	57.7 ± 17.6	0.293
HF <sub>RR</sub> (ms <sup>2</sup> )	666.3 ± 730.0	959.9 ± 914.7	0.238
HF <sub>RR</sub> (nu)	29.6 ± 21.6	33.8 ± 15.0	0.302
LF/HF	3.7 ± 2.8	2.4 ± 1.7	0.042
0 V (%)	28.1 ± 12.2	23.2 ± 11.1	0.031
2 V (%)	26.2 ± 11.3	30.5 ± 10.9	0.048
Resp. rate (breath/min)	16.3 ± 4.8	16.4 ± 3.1	0.940

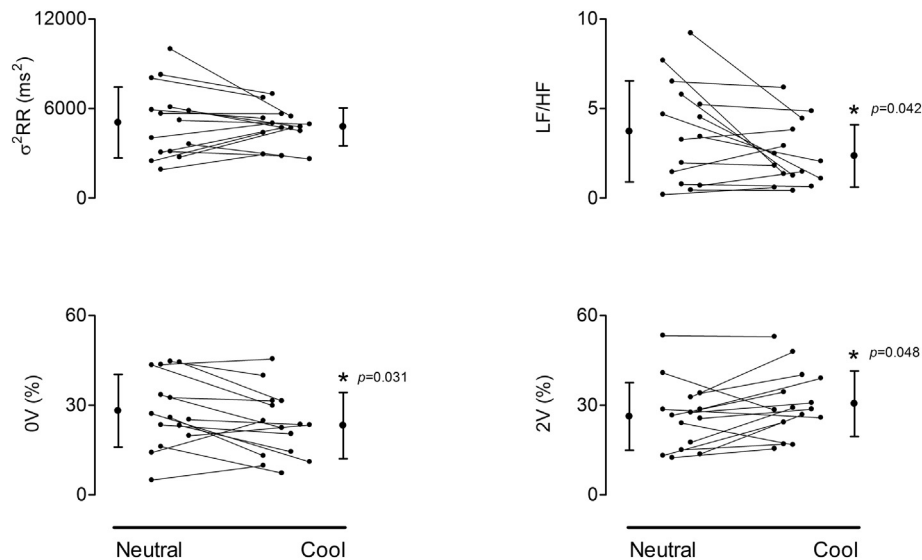
HR indicates heart rate; μ, mean; RR, R-R intervals; σ<sup>2</sup>, variance; LF, Low Frequency (≈ 0.1 Hz); nu, normalized units; HF, High Frequency (≈ 0.25 Hz); LF<sub>RR</sub> and HF<sub>RR</sub> are expressed in absolute values (ms<sup>2</sup>) and in normalized units (nu); 0 V, and 2 V symbolic indices of cardiac autonomic control (see text); Resp., respiration. Values are mean ± SD.

global cognitive performance in the presence of minimal thermal discomfort; 2) the greater cognitive performance observed during the COOL trial was associated with a lower cardiac sympathetic and greater cardiac vagal modulation compared to the reference condition.

#### 4.1. Cognitive performances and microclimate

Previous studies reported that the best cognitive performance, assessed both in working and learning activities, peaked at 21.6 °C, showing an inverted U-shape relationship with the indoor air temperature (Kosonen and Tan, 2004; Pilcher et al., 2002; Seppanen et al., 2006a, 2006b). Indeed, cognitive performance linearly decreased as the temperature increased above 25 °C and similarly decreased, when indoor temperature declined below 20 °C (Pilcher et al., 2002; Seppanen et al., 2003). However, while several studies described the effects of the higher indoor temperature on cognitive performance (first part of the inverted U-shape curve), there is insufficient data on the effects of cold exposure on performance (second part of the inverted U-shape curve) (Kosonen and Tan, 2004; Pilcher et al., 2002; Seppanen et al., 2006a, 2006b). The meta-analysis by Picher (Pilcher et al., 2002), suggested that both hot and cold temperatures negatively impact performance in a wide range of cognitive task. However, the results were significantly influenced by other variables different from the absolute value of the temperature, i.e., time of exposure to the temperature, task duration and type. These aspects make the relationship between indoor temperature and cognitive performance highly complex. As reported in a previous pilot study by our group (Barbic et al., 2020) the reduction of indoor temperature seemed to increase the cognitive performance in a group of students. However, the studies were performed in two days belonging to different periods of the year, meaning that they were characterized by significant differences in out-door temperatures. That unavoidably added a confounder making the interpretation of the results critical. Finally, the students' thermal comfort and the role of clothing were not addressed. Taken together these weaknesses prompted the planning of the present investigation.

In the current study we dichotomized the answers to the “Questionnaire for the Thermal Comfort Survey” to obtain a synthetic evaluation of the student's thermal feeling. This approach furnished the number and percentage of students reporting a feeling of “Thermal Comfort”. As expected, the percentage of students who reported a feeling of “Thermal Comfort” was higher during the Neutral compared to the Cool trial. However, during the Cool trial, none of the students answered “very uncomfortable”, only 4 answered “moderately uncomfortable” and 7 seemed to be only slightly disturbed by the temperature reduction (Table 2c). The Thermal Predicted Vote indicated that only 4 students would have required a “much warmer” temperature during the Cool trial, while all students were satisfied with humidity. These data indicated that students' global thermal comfort feeling



**Fig. 3.** Students' individual values and mean with standard deviation of RR interval variance ( $\sigma^2RR$ ) (upper left panel), spectral index of cardiac sympatho-vagal modulation LF/HF (upper right panel), and symbolic indices of cardiac sympathetic 0 V% and vagal 2 V% modulation (bottom panels) during the NEUTRAL and COOL trials. Notice that the spectral marker of cardiac sympatho-vagal modulation and the symbolic index of cardiac sympathetic control were significantly lower during the COOL compared to the NEUTRAL trial. Conversely, the symbolic index of cardiac vagal modulation was significantly higher during the COOL compared to the NEUTRAL trial. RR interval variance was similar during the two different trials.

was only slightly reduced during the Cool trial compared to the Neutral one. We hypothesized that during the cold season the students might be almost partially “habituated” to the low outdoor temperatures, thus allowing an easier adaptation to the microclimate of the Cool trial (Castellani and Young, 2016).

Differences in the clothing worn may influence cognitive processes (Adam and Galinsky, 2012; Hu and Maeda, 2020). Importantly, as the volunteers of the present study were unaware of the temperature set in the two different trials, they wore similar clothing, with the same clothing insulation. This limited the potential influence of different dressing habits on the results.

The present study suggests that, in the presence of a minimal thermal discomfort our students performed better in a cool compared with a neutral indoor environment, differently from what was reported by others (Kosonen and Tan, 2004; Pagani et al., 1991; Seppanen and Fisk, 2006; Seppanen et al., 2003). Differences in the duration and level of temperature exposure before and during the cognitive performance assessment, dissimilarities in the type and duration of the cognitive tasks, and different target populations, i.e. workers versus students, may account for the discrepancies (Kosonen and Tan, 2004; Pilcher et al., 2002; Seppanen et al., 2006a, 2006b). In addition, the effect of other “moderators” on mental performance such as the thermal acclimatization were only partially considered by other studies (Kosonen and Tan, 2004; Pilcher et al., 2002; Seppanen et al., 2006a, 2006b). On the other hand, our data are partially in keeping with what was recently observed by Hu and Maeda (Hu and Maeda, 2020) in a study aimed at evaluating productivity and physiological response during exposure to different air temperature. Indeed, these authors observed an increase of performance and productivity efficiency during cold exposure (16 °C) compared to a warmer condition. Also, they confirmed the importance of clothing in both thermal comfort and cognitive performance.

An important feature of the current investigation is that the above-mentioned variables, as well as age, gender, activity level, clothing and ventilation were kept similar on the two study trials (see Tables 1, 2). Notably, changes in each of these factors might have influenced cognitive performance (Jensen et al., 2009; Pilcher et al., 2002). Additionally, the similar and low values (i.e. <600 ppm) of indoor CO<sub>2</sub> measured during the lectures on NEUTRAL and COOL trials enabled us to exclude any role of CO<sub>2</sub> modifications potentially affecting the students' cognitive performance (Brink et al., 2020; Zhang and de Dear, 2017). It is essential to point out that

cognitive performance may be also influenced by outdoor temperature values (Galli et al., 2011) which may act as an additional powerful confounder, not even considered by previous studies (Pilcher et al., 2002). To overcome these potential limits, the trials of the current investigation were carried out during the cold season (December) in Italy. Importantly, this might have allowed the students' long-term acclimatization to cold outdoor temperatures, thus accounting, at least partially, for both the minimal thermal discomfort, and the significant improvement of the cognitive performance during the COOL trial.

In the present study, the order of the NEUTRAL and COOL trials was not randomized thus potentially raising the problem of a training effect leading to an artifactual improvement of the cognitive performance as far as the COOL trial was concerned. However, all the students underwent a previous, specific, training on the CBS test before starting the study protocol. Each student underwent several CBS tests trials to get familiar with that test modality. Importantly, the cognitive tests were randomly proposed with an equivalent level of complexity during the two trials. We believe that these methodological features ought to have significantly minimized any potential “learning” effect on CBS results during the COOL trial.

Finally, during the COOL trial, we observed a marked discrepancy between the nominal classroom temperature, set by the automatic conditioning system, and the actual temperature values recorded during the study day. In agreement with previous reports (Seppanen et al., 2006b; Zhang and de Dear, 2017), this finding highlights the importance of the continuous recording of indoor microclimate parameters independently of the automatic setting of the air conditioning system, particularly if any relationship between indoor temperature and humans' performance was to be assessed.

In the current study, among the different cognitive domains, only *reasoning* resulted unaffected by the two different classroom temperatures. This is in keeping with what was previously observed by our group (Barbic et al., 2019) and others (Lan et al., 2011; Pilcher et al., 2002; Siqueira et al., 2017), suggesting that the ultimate effect of indoor microclimate on cognitive performance was most likely dependent on a specific task.

#### 4.2. Cardiac autonomic profile and cognitive performance

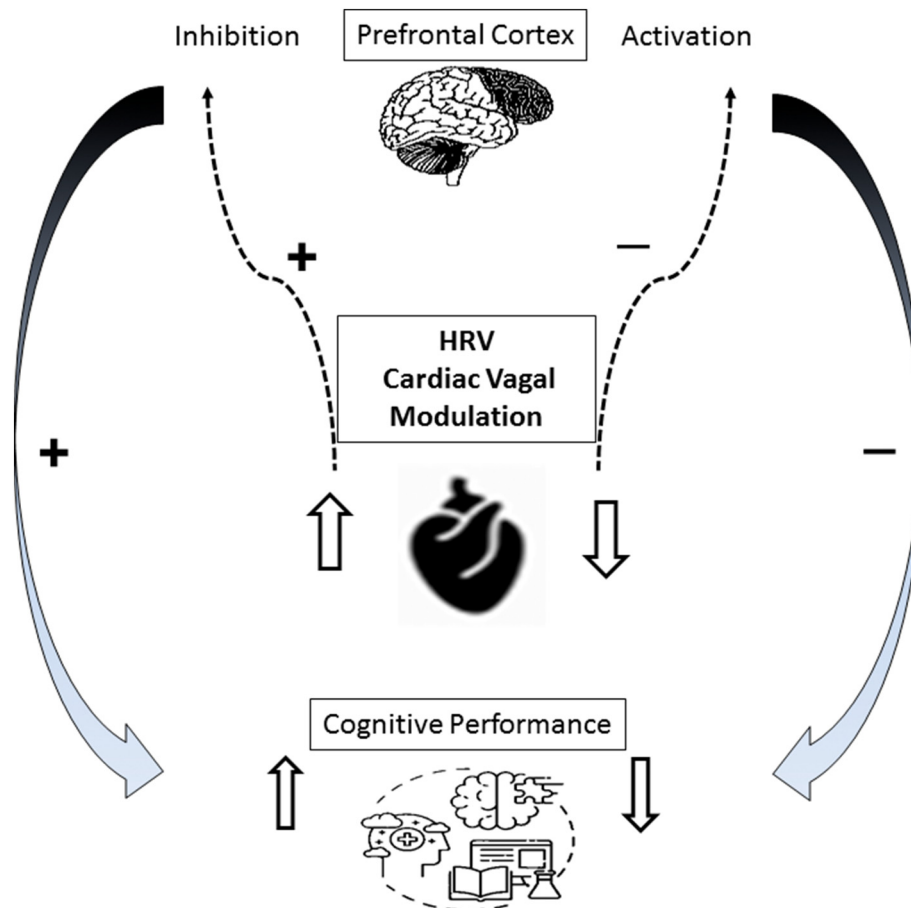
The students' cardiac autonomic profile assessment during NEUTRAL and COOL trials may furnish additional insights into the mechanisms

underpinning the observed differences in the cognitive performances. It is important to remind that the autonomic nervous system is largely involved in thermoregulation related cardiovascular changes (Liu et al., 2015; Sawasaki et al., 2001) and, on the other hand, undergoes significant modifications during mental tasks attendance (Giuliano et al., 2017; Hansen et al., 2004; Thayer et al., 2009). In addition, both linear and non-linear dynamics characterize the response changes of the cardiac autonomic control and these may require different computation methodologies to be properly addressed (Barbic et al., 2019; Guzzetti et al., 2005; Porta et al., 2007; Porta et al., 2015b). While spectral analysis of HRV has been widely used to study the reciprocal, and linear relationship between vagal and sympathetic cardiac modulation (Barbic et al., 2014b; Dalla Vecchia et al., 2013; Furlan et al., 1991; Furlan et al., 2000a), symbolic analysis does not require the hypothesis of linear dynamic to be fulfilled and, as such, can detect nonlinear interactions between sympathetic and vagal influences (Porta et al., 2015b). Situations in which sympathetic and vagal modulations did not produce linear additive effects to the sinus node may typically occur during the cortical activation involved during an attention task (Furlan et al., 2000a), including attending a lecture (Cacioppo et al., 2000).

In the present study, students showed the best cognitive performances during the COOL trial when their cardiac autonomic profile showed greater values of the symbolic index of cardiac vagal modulation (2 V%), and lower values of the symbolic index of cardiac sympathetic modulation (0 V%), compared to the NEUTRAL trial. Moreover, during the COOL trial the spectral index of cardiac sympatho-vagal modulation was reduced compared to the NEUTRAL one, pointing to an enhancement of the cardiovagal modulation. These observations are in agreement with the complex relationships

among the functioning of the prefrontal cortex involved in the cognitive performance, the central autonomic network and ultimately the sympatho-vagal balance modulating the heart period (Benarroch, 1993; Fuster, 2000a; Fuster, 2000b; Hansen et al., 2003; Hansen et al., 2004; Ter Horst and Postema, 1997; Thayer and Lane, 2009; Thayer et al., 2009). An inhibitory activity of the intact prefrontal cortex leading to prevailing cardiac vagal modulation seems to be mandatory for effective cognitive performance as described by Fuster and colleagues (Fuster, 2000a; Fuster, 2000b) and highlighted in Fig. 4. In addition, a greater heart rate variability and cardiac vagal modulation, as found, for example after physical training in healthy subjects, were associated with better management of some cognitive domains (Hansen et al., 2004; Luft et al., 2009). In keeping with these considerations, during the COOL trial we observed greater cognitive performance, greater values of the cardiac vagal modulatory activity indices and lower indices of sympathetic drive to the heart, compared to the NEUTRAL trial. However, we have to point out that no mechanistic insights could be found between the autonomic changes and modifications in cognitive performances indices.

The finding in the current study of an increased cardiac vagal modulation during the COOL trial, deserves additional comments, since one would expect a greater cardiac sympathetic and lower vagal activation in response to lower indoor temperatures according to previous investigations (Durand et al., 2004; Fagius and Kay, 1991; Stocks et al., 2004; Zhu et al., 2018). As reported above, the present study was carried out during the cold period in Italy, i.e., the month of December, when our students had undergone a prolonged cold exposure. In a study by Castellani and Young (2016) “habituation” is the most common pattern of thermoregulatory



**Fig. 4.** Interplay relationships between cardiac autonomic and cognitive performance. This picture shows the complex relationships between cardiac autonomic modulation cognitive performance and as it is mediated by the pre-frontal cortex functioning. As suggested by previous studies (Fuster, 2000a; Fuster, 2000b; Hansen et al., 2004; Thayer and Lane, 2009; Thayer et al., 2009) an increase of cardiac vagal modulation allows an increase of cognitive performance by sustaining an inhibition of the prefrontal cortex (left part of the picture). Conversely, a reduction of cardiac vagal modulation (i.e., a shift towards a prevailing cardiac sympathetic control), seemed to induce activation of the prefrontal cortex finally leading to a reduction in cognitive performance (right part of the picture).



adjustments observed in humans in response to chronic cold exposure. “Habituation” to low environmental temperature was found to be associated with a reduced sympathetic and enhanced parasympathetic activation during a new cold exposure (Harinath et al., 2005; Mäkinen et al., 2008), an experimental setting that we replayed during the COOL trial. These considerations may explain, at least partially, the enhanced vagal modulations observed in our students' group during the COOL trial. Also, thermal adaptation might account for the simple mild thermal discomfort that the participants of the present study complained of.

In the current real-life setting, we hypothesize that the chronic cold outdoor exposure of the students during winter could have induced a thermal adaptation to cold, resulting in a global better cognitive performance associated with an enhanced cardiac vagal modulation compared with the reference condition.

## 5. Strengths and limitations

In the present study, we sought to limit the possible effects of confounders characterizing a “real life” learning setting that might have altered the temperature perception and, consequently, might have blurred the link between cognitive performance and cardiac autonomic control in our population. Indeed, the same students participated in both the NEUTRAL and COOL trials. The lecture type and duration were similar during the two different trials. Cardiac autonomic profile was assessed in everyone after 40 min of adaptation to the indoor temperature. The present study was carried out in the students' habitual classroom, during a routine lecture aimed at acting as a mental load to emphasize possible cognitive weaknesses, if any, during the CBS. Finally, our results bear a potential ecological implication given that better cognitive performance was obtained in an indoor setting requiring a reduced energy expenditure (Table 2e).

Some limitations ought to be acknowledged. First, the target population of the present study was limited to 15 young and healthy volunteers. Therefore, results must be considered preliminary. In the present study, no crossover was performed as far as the order of the NEUTRAL and COOL temperatures trials was concerned. However, we believe that the potential “learning” effect during the cognitive test on the COOL trial is likely to be negligible, due to the specific training on CBS that students underwent before starting the study protocol. An additional limit of our study is related to the fact that the CBS evaluation test explored only few simple cognitive domains. Also, we did not consider the potential role of local thermal comfort of our students (i.e., the temperature variation between head and feet level) in influencing the cognitive performance. Due to all of these reasons, the results of the present study need to be further validated.

## 6. Conclusions

The results of the present study suggest that better cognitive performance was obtained during the cool temperature lecture performed at about 18.4 °C. This was associated with only minimal thermal discomfort with a cardiac autonomic profile characterized by a vagal predominance, compared to the reference condition.

These data may furnish new insights into the complex relationships among outdoor and indoor temperatures, students' cognitive performances, and cardiac autonomic control even if the involved mechanism still remain poorly understood. Also, results may have important practical implications if the attempt to optimize indoor temperature in teaching environments and workplaces is pursued. Indeed, the best cognitive performance reached by the students during the COOL trial lecture points to the possible beneficial effects of a reduction of classroom temperatures during the cold season to optimize learning as well as to possibly save energy.

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## CRediT authorship contribution statement

Conceptualization: F. Barbic, A. Porta, R. Gatti, R. Furlan; Investigation: F. Barbic, M. Minonzio, D. Shiffer, L. Cerina, S. Rigo, A. Bisoglio; Methodology: F. Barbic, R. Furlan, A. Porta; Formal analysis: F. Barbic, M. Minonzio, B. Cairo, L. Cerina, M. Vaglio, M. Santambrogio, S. Rigo, A. Bisoglio; Data curation: F. Barbic, M. Minonzio, B. Cairo, P. Verzeletti, F. Badilini, A. Porta, R. Furlan; Funding acquisition: R. Furlan, F. Barbic; Software: F. Badilini, A. Porta; Writing - original draft: F. Barbic, M. Minonzio; Writing - review & editing: F. Barbic, D. Shiffer, A. Porta, R. Furlan; Validation: F. Barbic, M. Minonzio, B. Cairo, D. Shiffer, L. Cerina, P. Verzeletti, F. Badilini, M. Vaglio, A. Porta, M. Santambrogio, R. Gatti, S. Rigo, A. Bisoglio, R. Furlan; Supervision: F. Barbic, R. Furlan, A. Porta.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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