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Effects of short-term exposure to moderate pure carbon dioxide levels on cognitive performance, health symptoms and perceived indoor environment quality

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ABSTRACT

Educational buildings frequently experience elevated CO_2 concentrations with inadequate ventilation and high occupancy, sometimes exceeding building guideline levels. Some studies reported detrimental impacts on cognitive performance of indoor CO_2 levels, while others did not. To generate further evidence, we conducted an experiment in an environmentally controlled chamber. Sixty-nine healthy university students were exposed individually for 70 min, in three separate sessions, to three CO_2 conditions of 600, 1500 and 2100 ppm (crossover design). With fixed ventilation rates, pure CO_2 was injected to achieve different exposure levels. A validated neurobehavioral BARS test battery was used to assess participants' cognitive performance. Participants gave subjective ratings of indoor environment and reported any health symptom through questionnaires. Comparing elevated CO_2 levels to 600 ppm, after adjusting for potential confounders, results showed significant improved performance, that is, responses were quicker in two out of ten tests, and no significant differences in accuracy for any test. Under 1500 ppm, participants rated the air quality significantly higher than at 600 ppm, but there were no differences at 2100 ppm. Differences were not significant on thermal sensation, perceived lighting quality, perceived noise level, or health symptoms for comparisons between conditions. Results indicate no clear link between pure CO_2 levels below 2100 ppm and cognitive performance, perceived indoor environment quality and health symptoms. The findings are consistent with some prior studies, indicating that pure CO_2 below 2100 ppm implies no harm in adults and should not be treated as a potential indoor pollutant in higher educational environments.

1. Introduction

Carbon dioxide has long played a role as a marker of bioeffluents, and its concentration level has been commonly used to guide ventilation practices for better indoor air quality in buildings. Human exposure to indoor carbon dioxide has increased over the years due to climate change, given the growth in atmospheric CO_2 concentration [1], whilst, on the other hand, ventilation rates have been drastically reduced for energy-saving reasons [2,3]. Raised concerns about the impacts of elevated CO_2 concentrations on performance in the educational environment have drawn lots of attention, as schools normally face the problems of increased occupancies and decreased ventilation rates. In the UK, the Department for Education and Skills provides a guidance standard document Building Bulletin 101 (BB101) for educational buildings [4], which recommends an average concentration of CO_2 should not exceed 1500 ppm and 2100 ppm with a minimum ventilation rate of 3L/s-p. Average levels of CO_2 concentrations normally range from 600 to 1000 ppm in the educational environment, sometimes may surpass 2000 ppm [5–7] and even reach a peak level of 4000 ppm [8,9].

Cognitive performance [10] is essential to people's daily life, domains such as memory, attention, concentration, motor skills, executive function, processing speed and verbal skills are linked to distinct regions of human brains for different functions. Cognitive performance measured by cognitive tests and learning outcomes quantified by academic records [6,11,12] showed decrements with inadequate air quality (characterized by CO_2 levels) in schools. In these studies, poor air quality due to insufficient ventilation rates might cause impairment in performance, and CO_2 was seen as a proxy for ventilation rates but not a

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potential pollutant.

However, being a proxy of ventilation effectiveness and an indicator of air quality, carbon dioxide itself started to gain attention in a limited number of studies about its direct impact on humans in the indoor environment. Some recent experimental studies (Table S1, supplementary material) investigated the association between elevated carbon dioxide, perceived indoor environment quality and self-reported health symptoms. According to EH40/2005 Workplace exposure limit [13], the long-term exposure limit of 8 h is 5000 ppm. Hence, negative consequences were observed even at exposure levels below the exposure

Table 1

Summary of previous studies exploring the effects of pure CO_2 on cognitive performance in built environment.

Study	CO_2 levels (ppm)	Ventilation rates	Subjects[a]	Environment	Time (h)	Cognitive Task	Effects of elevated CO_2	Response speed[b]	Accuracy[c]
Pure CO_2 addition with fixed ventilation rates									
Kajtar et al., 2012 [17]	E1:600,1500,2500,5000 E2:600,1500,3000,4000	33.3 L/s	Unknown occupations (E1: 10; E2: 10)	Laboratory room	E1: 2.33 E2: 3.5	Proofreading	E1: No effect E2: Reduced proofreading performance	No effect	600 vs 4000* (−)
Satish et al., 2012 [14]	600,1000,2500	24.85 L/s-p	University students (22)	Chamber	2.5	SMS	Reduced decision-making performance		
Allen et al., 2016 [15]	500, 1000, 1400	18.6 L/s-p	Office workers (24)	Lab with office environment	6.75	SMS	Reduced decision-making performance		
Zhang et al., 2016 [21]	500, 5000	33.3 L/s-p	University students (10)	Chamber	2.5	Text typing + Addition + Tsai-Partington	No effect	No effect	No effect
Liu et al., 2017 [22]	400, 3000	66.7 L/s-p	University students (12)	Chamber	3	Multiple tasks	No effect	No effect	No effect
Zhang et al., 2017 [28]	500, 1000, 3000,	33.3 L/s-p	University students (25)	Chamber	4.25	Multiple tasks	No effect	No effect	No effect
Allen et al., 2019 [32]	700,1500,2500	850 L/s	Pilots (30)	Flight simulator	3	FAA PTS	Reduced pilot performance as assessed by the examiner		
Snow et al., 2019 [27]	830, 2700	Background infiltration	University students (31)	Office room	1.97	CNS Vital signs battery	No effect	No effect	
Zhang et al., 2020 [24]	1500, 3500, 5000	8.68 L/s-p	University students (15)	Chamber	2	MATB	Reduced MATB task performance (system mentoring, tracking, scheduling and resource management)	MATB: 1500 vs 3500* (−)	
Pang et al., 2021 [25]	1500, 3500, 5000	8.68 L/s-p	University students (15)	Chamber	4	PVT + Cognitive tasks	Reduced vigilance	PVT: 1500 vs 5000* (−)	PVT: 1500 vs 3500* (−)
Tu et al., 2021 [19]	8000, 10000, 12000	0.5 L/s (Air purifier) + 0.052 L/s (O_2)	University students (30)	Chamber	4	Text typing + Numerical calculation	Reduced text typing performance	Text typing: 8000 vs 12000* (−)	No effect
Maniscalco et al., 2021 [20]	770, 20000	94.4 L/s	Workers (24)	Chamber	4	TAP	No effect	No effect	No effect
Cao et al., 2022 [26]	1500, 3500, 5000	8.68 L/s-p	University students (15)	Chamber	1.67	Multiple tasks	Reduced performance of visual attention, risky decision-making, and executive ability	VS, BART, Stroop: 1500 vs 5000* (−)	No effect
Pure CO_2 addition with varying ventilation rates									
Rodeheffer et al., 2018 [18]	600,2500,15000	Varying ventilation rates	Submariners (36)	Chamber	2.08	SMS	No effect		
Scully et al., 2019 [31]	600,1200,2500,5000	Varying ventilation rates	Astronauts (22)	Chamber	4	Cognition + SMS	Reduced performance on most decision-making measures, aggregate speed, accuracy and efficiency scores at 1200 ppm, compared to 600 ppm.	Overall speed: 600 vs 1200* (−), 1200 vs 2500* (+)	VOLT: 600 vs 2500* (+), 1200 vs 2500* (+), 2500 vs 5000* (−)

[a] The number in the bracket shows the sample size of the whole experiment or specific session.

[b] Effects of exposures to CO_2 on response speed, * means significant difference between the two exposure levels, (+) means response speed increased at higher CO_2 levels, (−) means response speed decreased at higher CO_2 levels.

[c] Effects of exposures to CO_2 on the accuracy, * means significant difference between the two exposure levels, (+) means accuracy increased at higher CO_2 levels, (−) means accuracy decreased at higher CO_2 levels.

limit. The adverse effects of pure CO_2 concentrations on cognitive performance such as decision-making [14,15] have been found at levels as low as 1000 ppm in the chamber and laboratory, while significant changes in physiological response [16] were reported from pure CO_2 levels at 3000 ppm and perceived air quality [17] from pure CO_2 levels of 3000 ppm. Due to specialised working environments such as underground confined spaces and submarines, some studies [18–20] examined the effects of exposure to indoor CO_2 concentrations above the occupational limit in the chamber, where CO_2 levels may build up to 20000 ppm. Detrimental impacts on health and text typing performance [19] were found when pure CO_2 concentrations elevated from 8000 to 12000 ppm.

Regarding the results of cognitive performance under elevated pure CO_2 levels (Table 1), the findings were inconsistent. Some studies reported significant decrements on performance, while others did not find significant results. There were three methods to control CO_2 levels in the studies: (1) injecting pure CO_2: most studies which used this method were conducted in the chamber [14,19–26] and controlled laboratory environment [15,17], one in the office room [27] (2) manipulating the ventilation rates (Table S2, supplementary material): studies used this method were conducted in chamber [28], open plan office [29] and classroom [30] (3) combining the two strategies: two studies were conducted in the chamber [18,31]. However, adjusting ventilation rates during the experiment would affect the concentrations of other indoor pollutants like bioeffluents [28] and contribute to cognitive performance decrement. The conflicting outcomes between the studies may be due to the diverse cognitive tests used in the experiment, differences in study populations, ventilation rates (pure CO_2 or mixed CO_2) and disparity in the experimental procedure.

When investigating CO_2 in its own right with fixed ventilation rates, pure CO_2 was added to achieve desired concentration levels in the chamber and laboratory room (Table 1). Significant effects of pure CO_2 on cognitive performance have been reported by some lab studies [14,15,17,19,24–26,32], while other studies found no statistically significant effects on cognitive performance during exposures [20–22,27,28].

The sensitivity, validity, and cognitive load of different assessment methods might have affected the results. Four studies used relatively simple tasks such as text typing and proof-reading [17,19,21,28], Kajtár et al. [17] did not find significant decrements until he increased the workload with a more challenging text in the second series of exposure. Zhang et al. [21,28] did not report any significant results in either of the two studies; besides these simple tasks, Zhang et al. [28] also used classical cognitive tests such as the Stroop test and the d2 test (measured attention and concentration), but no significant impacts were reported. Tu et al. [19] reported a significant decrease in typing performance, but at relatively high CO_2 concentrations of 12000 ppm, while the other three studies tested CO_2 levels below 5000 ppm. Two studies [14,15] used a computer-based test tool Strategic Management Simulation (SMS), to test participants' higher-order decision-making based on different proposed scenarios in the chamber [14] and in the lab with an office environment [15], both reported significant decrement in performance at CO_2 levels as low as 1000 ppm. Another assessment method with high mental load was used in a study conducted in a flight simulator [32], which suggested an adverse impact on pilot performance as assessed by examiner with lower passing rates at 1500 ppm, compared to 700 ppm. These findings indicate that when the compensatory mental effort was limited by the worse environmental conditions, like higher CO_2 levels, or under relatively higher cognitive loads of the tests, like the SMS test, the effects of CO_2 might be detected.

In terms of the experimental procedure and exposure durations, Kajtár et al. [17] suggested that longer exposure time, which compared 210 min–140 min, could ensure that participants wholly adapted to the experiment conditions. Whilst Kajtár et al. suggested exposure duration might play a critical role, studies with shorter and longer durations showed detrimental effects on cognitive performance. For studies that found significant effects and reported reduced performance at higher CO_2 levels, some employed a short exposure time of around 2–3h [14,24,32], Pang et al. [25] and Tu et al. [19] used 4h, and Allen et al. [15] used a much longer exposure time of 6.75h. Among the studies that did not find significant effects on cognitive performance, exposure time varied between 2 and 4.25h. Rodeheffer et al. [18] and Snow et al. [27] employed exposure of around 2 h, in the genesis hypo chamber and office room, and the other three studies [21,22,28] tested for longer exposure in the chamber. No significant changes in performance were reported during exposures of university students for 4.25h to CO_2 levels of 500,1000 and 3000 ppm [28], for 2.5h to CO_2 levels of 500 and 5000 ppm [21], and for 3h to CO_2 levels of 400 and 3000 ppm [22]. Considering the overlapping of exposure durations in studies that found effects and those that did not, the link between exposure time of pure CO_2 and changes in cognitive performance remains unclear. The minimum duration of exposure to CO_2 that may lead to an effect on people is still unknown, as many factors might have impact such as the space people stayed in, tasks involved and individual differences such as age, gender, and health state. However, continuous exposures above 2 h in working and learning environments are hard to maintain as breaks would occur. Evidence from shorter exposure durations is needed to understand the underlying mechanisms.

The disparity of participants may account for diverging outcomes in studies assessing CO_2 on cognitive performance. Previous research [33–35] has found that cognitive functioning is associated with people's occupations, as the mental loads demanded in jobs are different. In eight lab studies which reported significant decrements in cognitive performance, six studies [14,17,19,24–26] hired university students as participants, while three studies [21,22,28] did not find significant effects in university students. Two studies [15,32] that recruited various occupation participants other than students reported significant performance decreases at CO_2 levels below 2500 ppm. One hired working people [15] from different professions (i.e. technical, secretarial, managerial populations), and another one recruited pilots [32] due to the objective of the study.

Some studies examined the effects of pure CO_2 on participants' perceived air quality and self-reported health symptoms. Four studies measured perceived air quality (air freshness, acceptability of air quality) during the exposures. Only Kajtar's study reported that the

Table 2
Demographic characteristics of sixty-nine participants.

Category	Number of People	Percentage
Age		
18–20	8	11.6 %
21–23	20	29.0 %
24–26	17	24.6 %
27–29	13	18.8 %
30–37	11	15.9 %
Gender		
Female	37	53.6 %
Male	32	46.4 %
First language		
English	11	15.9 %
Indo-European family[a]	13	18.8 %
Sino-Tibetan family[b]	33	47.8 %
Austronesian family[c]	5	7.2 %
Dravidian family[d]	3	4.3 %
Other languages[e]	4	5.8 %
Education		
Bachelor	19	27.5 %
Postgraduate	35	50.7 %
PhD	15	21.7 %

[a] Indo-European family in this study included Bangla, French, German, Greek, Gujarati, Hindi, Portuguese, Romanian, Spanish and Swedish.

[b] Sino-Tibetan family in this study included Mandarin and Cantonese.

[c] Austronesian family in this study included Bahasa Melayu, Indonesian and Tagalog.

[d] Dravidian family in this study included Kannada, Malayalam, and Tamil.

[e] Other languages included Japanese, Korean and Turkish.

acceptability of air significantly decreased at elevated CO_2 levels of 3000, 4000 and 5000 ppm, compared to the baseline condition. The other three studies [21,22,28] found no effects on perceived air quality. The findings suggest that pure CO_2 is not perceptible at these levels. Eight studies investigated the impact on self-reported health symptoms, and only two studies [17,19] found fatigue was significantly increased when pure CO_2 levels increased from 600 to 3000 and 5000 ppm [17], as well as from 8000 to 12000 ppm [19]; other studies did not find any significant changes in self-reported health symptoms [20–22,25,27,28]. Overall, the results are inconsistent and limited evidence indicates that exposure to pure CO_2 levels below 5000 ppm may impact perceived air quality and self-reported health symptoms and generally limited data for levels below the BB101 threshold of 2100 ppm.

The inconsistent results from previous studies and the potential socioeconomic impact due to cognitive performance decrements in built environment stimulated this research project, conducted in an environmentally controlled chamber, to generate further evidence on the effects of pure CO_2 on cognitive performance, using a systematically structured valid test battery Behavioral Assessment and Research System (BARS) [36]. We hypothesized that exposure to elevated pure carbon dioxide concentration levels, independent of ventilation rates, will significantly decrease occupants' cognitive performance.

2. Methods

Using a crossover design (Table 3), participants were exposed to three CO_2 levels in an environmentally controlled chamber, with a fixed ventilation rate in each condition. During the exposure, participants' cognitive performance was assessed through a validated neurobehavioral test battery BARS on a laptop. In addition, participants gave subjective ratings of perceived indoor environment quality and reported uncomfortable symptoms such as headache, sleepiness, dizziness, fatigue, and breathing difficulty via questionnaires before and after the BARS test.

2.1. Facilities

The experiment was conducted in a stainless-steel environmentally controlled chamber, measuring 4.4 m wide × 4.6 m deep × 3.0 m high inside, with an internal volume of approximately 60 m^3. The chamber was away from exterior walls and was not affected by the local environment (heat, light, noise) outside the building. Through the chamber controller, the researcher accomplished precise control of the chamber environment, achieving defined temperature, humidity, CO_2 concentration levels and ventilation rates. Air circulation was realised through four fans, ensuring the air was well mixed across the whole chamber environment. Outdoor air was drawn through a HEPA filter from the building ventilation system by an inline duct fan and expelled into the chamber through a diffuser. The return air from the room exited through the diffused outlet and discharged into the building ventilation system. An air velocity sensor was fitted within the air inlet ductwork to measure the real-time ventilation rate, and transfer the data to controller simultaneously to allow the researcher to define the ventilation rate. Chemically pure carbon dioxide (99.8 %) was automatically drawn from a cylinder and well mixed with outdoor air to reach the desired test levels in the chamber environment. One sensor connected with the chamber control system was fixed 1.2 m high on the wall inside the chamber to monitor the real-time temperature, humidity and CO_2 level, with synchronised data displayed on the controller screen outside the chamber and signal feedback to activate the alarm if reaching a risk level. Another CO_2 monitoring and alarm system was configured as a backup system for an additional safety measure.

Table 3
Exposure sequences in the balanced order.

Exposure Sequence	Condition 1 (ppm)	Condition 2 (ppm)	Condition 3 (ppm)	Number of participant (percentage of total N)
1	600	1500	2100	11 (15.9 %)
2	600	2100	1500	11 (15.9 %)
3	2100	600	1500	12 (17.4 %)
4	2100	1500	600	12 (17.4 %)
5	1500	2100	600	12 (17.4 %)
6	1500	600	2100	11 (15.9 %)

Prior to the whole study (October 2021–March 2022), a daily purging process was conducted in the chamber for three months (July–September 2021). With the CO_2 cylinder closed, the chamber was thoroughly ventilated at the maximum ventilation rate for at least 4 h every day, to exclude stale air and residual emission from the chamber construction material. The surface of the chamber was carefully cleaned, complying with health and safety requirements under COVID-19. TVOC level and PM levels including PM2.5, PM10, PM Total were daily measured. Defined by particle size, PM2.5 and PM10 referred to size fractions which are below 2.5 and 10 μm. PM total referred to all particulate matter, regardless of size. The levels were low with an average TVOC level of 10 $\mu g/m^3$ and an average PM total level lower than 0.001 mg/m^3, which ensured good air quality before the start of the experiment.

2.2. Subjects

Participants were recruited from university students through email advertisements (sent to the internal email lists with permission obtained) and placed on campus and social media. Participants were selected according to the inclusion criteria: adults in a healthy state not taking medications; non-smokers; without any learning disorders like ADHD (attention deficit hyperactivity disorder), dyscalculia, dysgraphia, and dyslexia. They also had to confirm before each session that they had no COVID-19 symptoms. Each participant was reimbursed 40 pounds for taking part in all three sessions (5 pounds each time for the first two sessions and 30 pounds after the third session).

Du et al. [37] calculated the effect size of previous studies which focused on the effects of pure CO_2 on cognitive performance in their review paper. They found that the weighted effect size of cognitive results in most studies ranged between −1 and 1, except for the studies which used SMS battery [14,15] and large effects were reported. Among prior studies, only two studies provided effect size in sample size calculation [19,28] and both used 0.4. With this effect size, Zhang et al. [28] did not find effects of pure CO_2 on cognitive performance, while Tu et al. [19] reported detrimental effects. Lian et al. [38] provided a general reference for statistical power analysis in human health and productivity research, they calculated the effect size of multiple neurobehavioral tests (e.g. symbol digit, picture recognition and visual choice) used in former studies, and the average value was 0.24. Therefore, our study used an effect size of 0.25 for the sample size calculation and aimed to detect smaller effects which might be failed to be found in previous studies.

The sample size of this study was estimated using the G Power software [39,40]. Considering a within-subject experiment design with repeated measurements, a statistical power of 0.9; alpha error probability of 0.05; an effect size of 0.25 estimated from previous studies; correction among repeated measures chosen as 0.5 and non-sphericity correction to be 0.5 (based on the number of repeated measurements) [38], the sample size was estimated to be 58. Considering the possibility of dropout during the three-month experiment period, participants were over-recruited. During the recruitment phase, 79 students expressed their interest in the study and registered for the first testing session, eight students dropped out during the first experiment session and two students dropped out during the second experiment session. In total, 69 participants (Table 2) completed all three experiment sessions, allowing the detection of an effect size of 0.23. According to Cohen's f guidelines

[41], f = 0.10–0.25 corresponded to a small effect, f = 0.25–0.40 to a medium effect, and f > 0.40 to a big effect. Therefore, the effect size f < 0.25 indicated that the sample size could detect a small effect of carbon dioxide in our study.

2.3. Experimental procedure

The study started in October 2021 and ended in March 2022. Each participants visited the environmentally controlled chamber at UCL Here East three times, each time exposed to one CO_2 condition and lasted 70 min. As the study was conducted during COVID-19, UCL required that masks must be worn on campus, including offices and labs, except working alone in single-occupancy offices. For the purpose of this research, participants wearing masks in the chamber might have impact on the study [42] due to possible elevation of carbon dioxide rebreathing from the masks, thus participants were tested alone each time in the chamber, without masks. There were four time slots every day for participants to choose from: 9:00–10:10, 11:30–12:40, 14:00–15:10, and 16:30–17:40. Each participant chose one time slot on one day to complete one experiment session in one month and 40.6 % participants chose same time slots across three sessions. The interval between two experiment sessions for one participant was at least four weeks, to minimize the learning effects of cognitive tests, and 30.4 % participants chose same weekday across three sessions. Participants received a reminder email with essential information 24 h before each experiment session and an information sheet was attached for the first session. Participants were advised to ensure adequate sleep before the test day, avoid intense physical activity for at least 12 h prior to the experiment, avoid coffee, sodas, energy drinks, chocolate and non-essential medications containing caffeine and refrain from using strong-scent perfume.

On the day of the experiment, participants came to the chamber at the scheduled time and stayed inside (Fig. 1) for about 70 min (Fig. 2). In the first experiment session, the introduction session lasted for about 10 min. The researcher briefly introduced the experiment to the participant and answered related questions. After reading the information sheet and confirming all essential information, the participant gave written and informed consent to the participation. The introduction session was usually shorter in the second and third sessions as participants already had some knowledge about the experiment, lasting about 5 min for readdressing important tips and signing consent forms. After the introduction session, the researcher left the chamber and the participant stayed inside alone for the following part. The participant was suggested first to do some quiet non-work-related activities (e.g. reading novels, watching videos, listening to music, sketching, browsing social media) to adapt to the chamber environment, for about 20 min. When the time was up, participants were instructed to fill in the pre-assessment questionnaire which included demographic details, subjective ratings of the perceived indoor environment quality and reporting any uncomfortable symptoms. Then the participant put on the noise-cancelling headphones and started the BARS test on the dedicated laptop, lasting about 40 min. After the cognitive test, the participant filled in a post-assessment questionnaire, which again rated perceived indoor environment quality and reported any health symptoms at the end of the experiment before leaving the chamber.

Fig. 1. Image of the chamber and experiment setup.

Ethical approval

This study was reviewed and approved by the UCL BSEER Research Ethics Committee (project ID: 20201027_IEDE_PGR_ETH; risk assessment ID: RA042087/2). Participants were informed of the objective of the research and provided assurances that their data would be kept confidential and secured, with project registration obtained from UCL Data Protection Office (Reference No Z6364106/2019/12/77). During the experiment, participants used subject IDs (code names) to keep their information anonymous (specific rules were given to generate their subject IDs). The subject ID was used in both the questionnaire and cognitive test. Participants were allowed to withdraw from the experiment at any time without giving a reason.

2.4. Exposure conditions

Every participant was exposed alone in the chamber to three different conditions of CO_2 levels: 600, 1500 and 2100 ppm. Participants were blinded to the experimental conditions and randomly participated in one of the six balanced-order sequences (Table 3). Under a high ventilation rate of 108 m^3/h (30 L/s-p), CO_2 was reduced to the background levels at the baseline condition, which was around 600 ppm with one person inside the chamber. To reach higher CO_2 levels in the other two exposure conditions, chemically pure CO_2 (99.8 % in quality) was automatically injected into the chamber from the cylinder. As this study focused on the educational building environment and university students, 1500 ppm was chosen as the CO_2 exposure level of the intermediate condition corresponding to the recommended daily average concentration levels in Building Bulletin 101 (2018) [4]. The highest exposure level was 2100 ppm, corresponding to the minimum threshold for ventilation rates of 3 l/s/p under a steady state in BB101-2018 [43]. The ventilation rate, temperature (23 °C) and relative humidity (50 %) were kept constant in all three conditions. The temperature of 23 °C was chosen corresponding to the recommended temperature for classroom 20.5–25.5 °C in ASHRAE Standard 55 (2020) during winter [44] and relative humidity of 50 % corresponded to the recommended relative humidity 40%–70 % by HSE [45]. With noise-cancelling headphones on, noise levels were maintained low during the exposures.

2.5. Measurements

Two environmental sensors continuously monitored the CO_2 concentration levels, temperature and relative humidity of the chamber environment during the exposures. One was the Rotronic CF1 measurement transmitter, connected with the chamber control system, fixed 1.2 m high on the wall, transferring the real-time monitoring data back to the chamber controller and presented on the touchable screen. The other one was a movable TESTO 480 monitor placed at 1.2 m high in the chamber to ensure the indoor air was well mixed with artificial CO_2 during the experiment. In addition, a PhoCheck Tiger PID monitor which was calibrated with isobutylene continuously measured the TVOC levels and a DustTrak DRX aerosol monitor for the PM levels (PM1, PM2.5, PM10 and PM total). Measurement of illuminance level on the desk was conducted with a Konica Minolta T-10A illuminance meter. The sound level inside the chamber was measured with TENMA 72–947 sound meter. A noise cancelling headphone was used to minimize the impacts of noise and provide a robust sound environment for the participant. The active noise cancellation system of the headphone reduced the incoming sound by 20–25 dB. The technical specifications

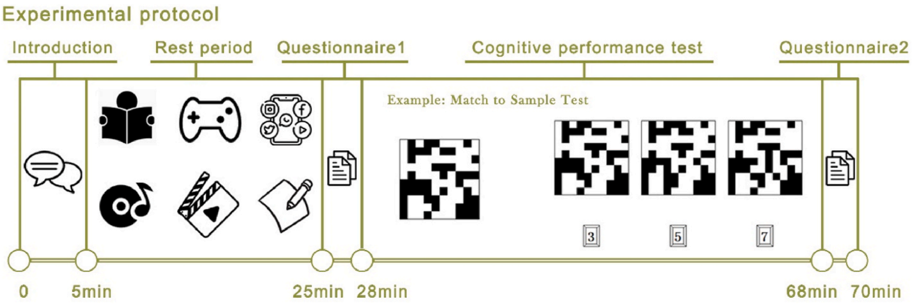

Fig. 2. Diagram of the experimental protocol.

of all monitors provided by manufacturers were listed in Table S3. Prior to the experiment, all monitors and the environmental chamber were calibrated.

As a computerised assessment system designed to evaluate the neurobehavioral function of individuals, the BARS test battery [36] has been used to study the effects on people's neurobehavioral performance with exposures to hydrogen sulfide gas, toluene, pesticide, and cadmium [46–51]. In this study, ten tests [52] (details see Table 4) were selected to assess participants' cognitive performance during exposure to different CO_2 concentration levels: match to sample (MTS), continuous performance test (CPT), symbol digit test (SDT), tapping (TAP), simple reaction test (SRT), reversal learning test (RLT), selective attention test (SAT), digit span (DST), serial digit learning (SDL) and progressive ratio test (PRT). To minimize order effects, the test battery was applied in a balanced order of ten sequences (Table S4, Supplementary Material). Each participant was tested in three different sequence orders across three conditions. Considering the repeated measures in the experiment design, learning effects may occur and parallel test settings in BARS battery could mitigate this problem. Similar but different tests were generated each time based on the parameters randomly changed within the setting range, such as intervals between trials, different stimuli, varied patterns and orders.

To obtain the subjective assessment from the participants, they filled in questionnaires on acute health symptoms and perceived indoor environmental quality before and after the BARS test. In both the pre-assessment and post-assessment questionnaires, participants rated the quality of the indoor environment of the chamber, including thermal sensation, indoor air quality, lighting quality, noise level, and reported any acute health symptoms like headache, sleepiness, dizziness, fatigue, and breathing difficulty. Thermal sensation was evaluated with the ASHRAE seven-point scale (ASHRAE Standard 55–2020) [44]. Perceived indoor air quality, lighting quality, noise level and self-estimated difficulty level of the test were assessed on a similar seven-point Likert scale, e.g., for air quality, ratings included "extremely bad (−3)", "bad (−2)", "slightly bad (−1)", "neutral (0)", "slightly good (+1)", "good (+2)" and "extremely good (+3)". Additionally, the pre-assessment questionnaire collected demographic information (gender, age, educational background, and first language) about the participants. Participants needed to provide some details of activities relevant to this study: consumption of breakfast, coffee, tea, or energy drinks before the test, whether they engaged in any strenuous physical activity during the previous 2 h, how long they slept the night before, whether the female participants were in menstrual period, and their clothing level [44] during the experiment with a checklist. The post-assessment questionnaire included their self-estimated difficulty level of the test and whether their ability to accomplish the test was affected due to personal reasons.

Table 4
Cognitive domains tested and parameter settings in the BARS battery.

Neurobehavioral test	Cognitive domain	Parameter setting
Match-to-Sample (MTS)	Visual memory	Number of trials, patterns[a], durations, intervals[a]
Continuous Performance Test (CPT)	Sustained visual attention	Number of trials, durations, stimuli[a], distractors[a]
Symbol Digit Test (SDT)	Attention, motor speed	Orders[a], durations
Tapping (TAP)	Motor speed, coordination	Durations, trial sequences[a]
Simple Reaction Time (SRT)	Motor speed	Stimulus, number of trials, intervals[a]
Reversal Learning Test (RLT)	Learning	Number of trials[a], stimulus, sequences[a], durations
Selective Attention Test (SAT)	Sustained attention	Number of trials, durations, intervals[a], sequences[a]
Digit Span (DST)	Attention, memory	Span type, trial sequences[a], durations
Serial Digit Learning (SDL)	Learning	Durations, trial sequence[a]
Progressive Ratio (PRT)	Motivation	Durations

[a] Parameters that randomly changed within a defined range each time to generate parallel tests.

2.6. Statistical analysis

Descriptive analysis was performed for the chamber's measured conditions, the BARS test results from two perspectives, response time and error rate, and means and standard deviations were reported. Due to the repeated measurements (same participant under different chamber conditions), univariable linear mixed-effect models were used to assess the association between cognitive test results (response times and error rates) and CO_2 concentrations (600, 1500 and 2100 ppm). Potential confounding factors (Table S5, Supplementary Material) that were considered in this analysis were: gender [53–57], age [54,55,57–60], education [53,57], first language [61], weekday [62,63], test time durations (41.5 ± 4.6 min), meal [64], caffeine drink [65], exercise [66], sleep hours [67], thermal sensation [68], perceived air quality [69], perceived lighting quality [70], perceived noise level [70,71], acute health symptoms [72–75], personal impacts, menstrual period [76], clothing level, perceived difficulty level [77], exit, TVOC concentration [15,78] and PM levels [79]. Factors that were significantly associated (p-value <0.05) with at least five cognitive performance outcomes (response times and error rates of BARS tests) across the three conditions were then included in multivariable mixed-effect models to correct for confounding. Similarly, to explore the effects of CO_2 levels on the perceived indoor environment quality and health symptoms, univariable linear mixed-effect models were used to assess the associations. The analysis was performed using IBM SPSS Statistics (version 28.0.0.0), and a p-value <0.05 was considered statistically significant.

3. Results

3.1. Measured conditions of the chamber environment

Table 5 presents the conditions measured in the environmental chamber during exposures. The average CO_2 concentration levels in the baseline, intermediate and high conditions were 633, 1520 and 2120

Table 5

Measured conditions in the environmental chamber (mean ± standard deviation).

Condition	CO_2 (ppm)	Temperature (°C)	RH (%)	Ventilation rates (m^3/h)	TVOC (μg/m^3)	PM2.5 (μg/m^3)	PM10 (μg/m^3)	PM Total (μg/m^3)
1	633 ± 64	23.2 ± 0.1	50 ± 1	108.1 ± 0.4	110 ± 99	0.2 ± 0.6	0.2 ± 0.6	0.4 ± 0.7
2	1520 ± 27	23.2 ± 0.1	50 ± 1	108.0 ± 0.2	116 ± 105	0.2 ± 0.5	0.2 ± 0.5	0.3 ± 0.6
3	2120 ± 36	23.2 ± 0.1	50 ± 1	107.9 ± 0.3	105 ± 103	0.2 ± 0.4	0.3 ± 0.5	0.4 ± 0.6

ppm, described as 600, 1500 and 2100 ppm in the paper. The results showed that CO_2 levels were effectively controlled within 64 ppm of the desired exposure levels in the baseline condition, whereas CO_2 levels in the other two conditions were maintained within 36 ppm of the desired exposure levels. The temperatures were controlled to values varying within 0.1 °C–23 °C, with mean values of relative humidity varying within 1 % of 50 %. Mean ventilation rates were all varied below 0.2 m^3/h (from 107.9 to 108.1 m^3/h) and were maintained to within 0.4 m^3/h of the average values. This study continuously measured the levels of two indoor pollutants during exposures, total volatile organic compounds (TVOC) and particulate matter (PM). The WHO Air Quality Guidelines for Particulate Matter, Ozone, Nitrogen Dioxide and Sulfur Dioxide (2021) suggests that average daily and annual limits of fine PM2.5 should not exceed 5 and 15 μg/m^3 and for PM10 are 15 and 45 μg/m^3 [81]. The results showed that the TVOC concentrations were low and PM levels (PM2.5 and PM10) during the exposures were below the guideline limits. The average lighting level of the test area was 365 lux and the noise level in the chamber was 60 dB. With the active noise cancellation system of the headphones, the incoming background noise for participants was reduced by 20–25 dB, hence the actual noise level to which participants were exposed during the experiment was 35–40 dB. Participants were advised to keep a thermal neutral state in the chamber, therefore they could adjust their clothing accordingly during the test, with the average CLO value being 0.77 ± 0.28.

3.2. Questionnaire results

The pre-assessment questionnaires indicated that 79.7 % of the participants had meals before the experiment at baseline condition, the proportions at the higher CO_2 levels of 1500 ppm and 2100 ppm were 88.4 % and 82.6 % respectively. Regarding consumption of coffee, soda and energy drinks, the proportions of participants who drank any of these were 7.2 %, 13.0 % and 13.0 % at three conditions. 2.9 %, 7.2 % and 4.3 % of participants had intense physical activity 2 h before the experiment session, separately. Despite recommendations to ensure adequate sleep before the experiment day, 26.1 % of participants slept less than 7 h before the baseline condition. The proportion of inadequate sleep for the other two exposure levels were 18.8 % and 27.5 %. In the baseline condition, 10.8 % of female participants reported having their menstrual period, with 16.2 % in both elevated CO_2 conditions. Fig. 3 shows the subjective ratings of the perceived difficulty level for the BARS test. The majority of participants rated the difficulty level between "very difficult" and "slightly easy", and nearly half of the participants gave ratings of "difficult" (very difficult and slightly difficult) at the three conditions: the proportions were 49.2 %, 46.3 % and 47.8 %, indicating that the BARS test used in this study having enough cognitive load to measure the changes of cognitive performance in these participants.

3.3. BARS test results

Figure S1 (Supplementary Material) shows the means for response times (a) and error rate (b) in all ten BARS tests at each CO_2 exposure level. Participants performed faster in six tests (MTS, CPT, PRT, DST, SRT and SAT) at higher CO_2 levels, compared with baseline condition, while participants' response times increased in the reversal learning test (RLT) when CO_2 levels increased. Participants performed faster in the symbol digit task at the intermediate level of 1500 ppm, compared with the baseline and highest conditions. In contrast, participants performed slower at 1500 ppm in serial digit learning and tapping test, compared with the other two conditions. With regard to the accuracy performance, discrepancies in trends were reported in ten tests. For reversal learning and symbol digit test, the percentage of error increased with CO_2 levels elevated from 600 to 2100 ppm. Compared with the baseline and highest conditions, participants performed better in the match to sample and digit symbol tests at 1500 ppm, while worse performances were shown at 1500 ppm in the other four tests, continuous performance test, tapping, selective attention and serial digit learning. As for the progressive ratio test and simple reaction test which mainly focused on assessments of individuals' motor speed, the error rates were relatively stable across three conditions.

Table S6 (Supplementary Material) illustrates results from the univariable mixed-effect models assessing the associations between cognitive test results and the potential confounding factors mentioned earlier. After identifying the factors with statistically significant associations with at least five BARS performance outcomes, nine factors (gender, age, first language, weekday, test durations, time slots, perceived air quality (before cognitive test), perceived noise level and perceived difficulty level) were selected as covariates for the multivariable mixed-effect models. Although in this study, gender showed no significant effects

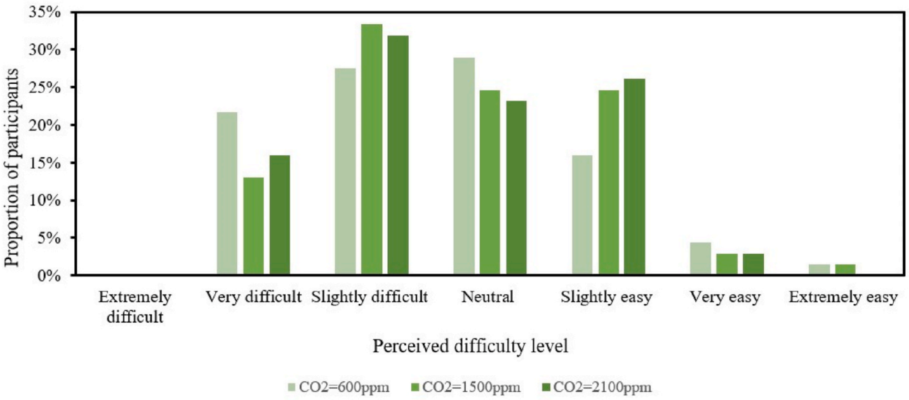

Fig. 3. Participants' perceived difficulty level of the BARS test during exposures to the three CO_2 conditions.

on the test results, it was still used in the multivariable model as a potential confounder since many studies [53–57] found that gender could impact cognitive performance.

After adjusting for potential confounders, only a few of the cognitive performance outcomes demonstrated significant effects (Table 6). Regarding response times, six of ten tests indicated a trend for reduced response time at elevated CO_2 levels, but only two found statistically significant decreases (Fig. 4). For the Selective Attention test, in the univariable models, the association of CO_2 levels with response times was not statistically significant at 1500 ppm compared to 600 ppm (p-value = 0.08), only the difference of response times between CO_2 levels of 2100 ppm and 600 ppm was statistically significant (p-value = 0.004). In the multivariable models, the response times significantly decreased at 1500 ppm (p-value = 0.03) and 2100 ppm (p-value = 0.002), compared to the baseline condition. For the Progressive Ratio Test, in both the univariable models and the multivariable models, significant effects of elevated CO_2 levels on response time were found at 1500 ppm and 2100 ppm. Regarding the error rates of cognitive tasks, no significant effect on accuracy was found in any of the ten BARS tests.

3.4. Perceived indoor environment quality and acute health symptoms

The mean value for subjective ratings of perceived air quality, perceived lighting quality, thermal sensation and perceived noise level across all conditions fell around the scale's midpoint (0 on a scale from −3 to +3), demonstrating a neutral perception of the indoor environment quality in the three conditions. The subjective assessment of the indoor air quality, thermal sensation and acute health symptoms under three CO_2 levels reported by participants have been presented in Fig. 5. Among all parameters of perceived indoor environment quality (Table S7, Supplementary Material), perceived air quality before the BARS test (η^2 = 0.017) was the only one that differed significantly

Table 6

Univariable and multivariable associations of conditions with response times and error rates in the ten BARS tests (MTS- Match to Sample, CPT- Continuous Performance Test, PRT- Progressive Ratio Test, SDT- Symbol Digit, SDL- Serial Digit Learning, TAP- Tapping, DST- Digit Span, SRT- Simple Reaction, SAT- Selective Attention Test, RLT- Reversal Learning).

CO_2 levels (ppm)	Response times of BARS tests				Error rates of BARS tests			
	Univariable models		Multivariable models[a]		Univariable models		Multivariable models[a]	
	β- coeff. (95 % CI)	p-value	Adj.β- coeff. (95 % CI)	p-value	β- coeff. (95 % CI)	p-value	Adj.β- coeff. (95 % CI)	p-value
MTS								
600	Ref.		Ref.		Ref.		Ref.	
1500	−11.06 (−190.66, 168.55)	0.90	49.48 (−126.33, 225.29)	0.58	−1.21 (−4.60, 2.18)	0.48	−1.12 (−4.46, 2.23)	0.51
2100	−36.81 (−256.81, 183.19)	0.74	28.58 (−178.88, 236.04)	0.79	−1.01 (−4.88, 2.86)	0.61	−1.22 (−4.93, 2.48)	0.52
CPT								
600	Ref.		Ref.		Ref.		Ref.	
1500	−1.58 (−11.59, 8.43)	0.76	0.92 (−8.89, 10.74)	0.85	0.41 (−0.54, 1.35)	0.40	0.03 (−0.90, 0.97)	0.94
2100	−8.58 (−21.55, 4.39)	0.19	−8.84 (−21.28, 3.59)	0.16	−0.16 (−1.23, 0.90)	0.76	−0.26 (−1.29, 0.77)	0.62
PRT								
600	Ref.		Ref.		Ref.		Ref.	
1500	−4.80 (−8.87, −0.72)	**0.02**	−4.14 (−8.09, −0.19)	**0.04**	−0.001 (−0.003, 0.001)	0.51	−0.001 (−0.003, 0.001)	0.42
2100	−6.10 (−11.54, −0.66)	**0.03**	−6.65 (−11.76, −1.54)	**0.01**	−0.001 (−0.004, 0.001)	0.26	−0.002 (−0.004, 0.001)	0.20
SDT								
600	Ref.		Ref.		Ref.		Ref.	
1500	−6.13 (−59.81, 47.55)	0.82	1.94 (−46.20, 50.07)	0.94	−0.06 (−0.98, 0.85)	0.89	−0.13 (−1.04, 0.78)	0.78
2100	2.04 (−68.37, 72.46)	0.95	23.72 (−37.38, 84.82)	0.45	0.61 (−0.39, 1.61)	0.23	0.46 (−0.50, 1.41)	0.35
SDL								
600	Ref.		Ref.		Ref.		Ref.	
1500	21.45 (−285.85, 328.75)	0.89	44.60 (−261.82, 351.03)	0.77	3.06 (−1.08, 7.20)	0.15	3.65 (−0.38, 7.68)	0.08
2100	−70.72 (−433.05, 291.60)	0.70	43.57 (−288.26, 375.40)	0.80	1.77 (−3.38, 6.93)	0.50	2.10 (−2.65, 6.86)	0.38
TAP								
600	Ref.		Ref.		Ref.		Ref.	
1500	−0.33 (−29.94, 29.27)	0.98	7.51 (−20.56, 35.58)	0.60	0.66 (−0.11, 1.44)	0.09	0.47 (−0.31, 1.25)	0.24
2100	−1.59 (−38.56, 35.37)	0.93	5.58 (−28.85, 40.01)	0.75	−0.03 (−0.87, 0.82)	0.95	−0.05 (−0.87, 0.77)	0.90
DST								
600	Ref.		Ref.		Ref.		Ref.	
1500	−134.68 (−450.27, 180.91)	0.40	−52.00 (−357.17, 253.17)	0.74	−1.49 (−3.37, 0.39)	0.12	−1.49 (−3.37, 0.38)	0.12
2100	−193.90 (−558.69, 170.89)	0.30	−75.44 (−420.06, 269.18)	0.67	−0.93 (−3.20, 1.34)	0.42	−0.55 (−2.67, 1.57)	0.61
SRT								
600	Ref.		Ref.		Ref.		Ref.	
1500	−0.65 (−19.21, 17.91)	0.95	−0.95 (−19.73, 17.84)	0.92	−0.003 (−0.019, 0.013)	0.72	−0.007 (−0.024, 0.010)	0.38
2100	−8.97 (−30.22, 12.28)	0.41	−9.16 (−29.85, 11.53)	0.38	0.005 (−0.012, 0.021)	0.58	0.004 (−0.013, 0.020)	0.66
SAT								
600	Ref.		Ref.		Ref.		Ref.	
1500	−8.88 (−18.73, 0.96)	0.08	−11.29 (−21.11, 1.47)	**0.03**	0.45 (−1.91, 2.81)	0.71	0.77 (−1.63, 3.19)	0.53
2100	−17.57 (−29.57, −5.56)	**0.004**	−17.59 (−28.90, −6.29)	**0.002**	−1.41 (−3.95, 1.13)	0.27	−1.41 (−4.04, 0.76)	0.18
RLT								
600	Ref.		Ref.		Ref.		Ref.	
1500	100.26 (−130.39, 330.91)	0.39	151.85 (−60.44, 364.14)	0.16	0.22 (−2.51, 2.95)	0.88	0.46 (−1.52, 2.44)	0.65
2100	6.81 (−221.50, 235.12)	0.95	68.45 (−139.16, 276.06)	0.52	1.51 (−1.74, 4.76)	0.36	1.94 (−0.40, 4.27)	0.10

[a] Model adjusted for gender, age, first language, weekday, test durations, time slots, perceived air quality (before BARS test), perceived noise level and perceived difficulty level.

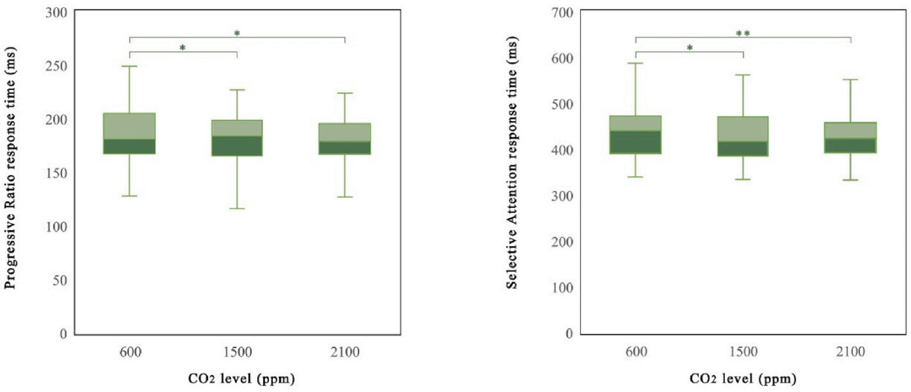

Fig. 4. Response times of Progressive Ratio test and Selective Attention test at three CO_2 exposure levels of 600, 1500 and 2100 ppm. $^*p < 0.05$, $^{**}p < 0.01$.

between CO_2 exposure conditions. Participants gave significantly higher ratings of perceived air quality at intermediate exposure levels of 1500 ppm, compared with 600 ppm (adj.β-coef. 0.28, 95 % CI (0.003, 0.547), p-value = 0.047), but no statistically significant differences at 2100 ppm compared to 600 ppm (adj.β-coef. −0.04, 95 % CI (−0.37, 0.28), p-value = 0.79). For perceived air quality after the BARS test ($\eta^2 = 0.001$), thermal sensation before ($\eta^2 = 0.003$) and after the BARS test ($\eta^2 = 0.009$), perceived lighting quality before ($\eta^2 = 0.004$) and after the BARS test ($\eta^2 = 0.002$), or perceived noise level ($\eta^2 = 0.003$), no significant effect of CO_2 levels was found. No significant effects of CO_2 levels on headache, sleepiness, dizziness, fatigue and breathing difficulty were reported.

4. Discussion

The findings of this study indicate that exposures to pure elevated CO_2 levels up to 2100 ppm were not associated with detrimental changes in individuals' cognitive performance in eight out of ten tests (match to sample, continuous performance, symbol digit, tapping, simple reaction, reversal learning, digit span and serial digit learning), perceived indoor environment quality and acute health symptoms, while Selective Attention and Progressive Ratio tests reported significantly improved performance with reduced response time at 1500 and 2100 ppm, compared to a baseline of 600 ppm. Our results deviated from some previous studies that indicated detrimental effects of pure CO_2 on cognitive performance and health symptoms [14,15,17,19,24–26,32], and were consistent with those that found no effects [20–22,28].

Compared with most studies which found adverse effects of pure CO_2 on cognitive performance, our study used relatively lower CO_2 levels, independent of ventilation rates. Some studies [17,19,24,25] testing higher CO_2 concentrations exceeding 3000 ppm tended to be more likely to exhibit the effects of CO_2 on individuals. The principal aim of this study was to examine the effects at levels close to routine scenarios in the educational environment, assessing the two thresholds in Building Bulletin 101, 1500 ppm as the intermediate condition and 2100 ppm as the highest condition.

For previous studies using similar exposure conditions of pure CO_2 up to 3000 ppm, disparities in the results might be partly due to the different population groups. Our study was consistent with the two studies which found no effects on university students [22,23], contrary to Allen et al. which focused on office workers [15] and pilots [32]. However, Satish et al. [14] found effects in university students at levels as low as 1000 ppm. One possible explanation for the discrepancies among the findings could be attributed to the different cognitive assessment methods utilised and varied cognitive domains measured. Except for the study using a particular subgroup of airplane pilots, Allen et al. and Satish et al. both found decrement in decision-making performance with the SMS battery, while our study with the BARS battery and the other two studies with combined cognitive tasks did not report effects of pure CO_2 concentrations. Less than 5.8 % of participants rated the BARS test as very easy and extremely easy at three conditions, and an average of 22.2 % of participants rated the difficulty level as slightly easy (Fig. 3), which proved the difficulty level of the BARS test was enough for most university students participated in this study. The SMS battery was described as a sensitive cognitive function assessment tool, but limitation also exists, as Rodeheffer et al. [18] mentioned. As a commercialised and proprietary battery, the details of the outcome calculation method were not transparent to users, for example, typical measurements such as response times and error rates were not present in studies which used SMS. The relationship with cognition domains was unclear, too.

For the majority of tests used here, our findings concur with studies reporting null effects on response time when exposed to elevated pure CO_2 levels below 5000 ppm [17,21–23]. Zhang et al. [82] found that the beta relative power of EEG significantly increased at 5000 ppm, compared to 1500 ppm, indicating that elevated CO_2 levels could be associated with higher arousal. Zhang et al. [21,28] postulated a weak indication of arousal increased at higher CO_2 levels, based on the slightly increased $ETCO_2$ and reduced performance of Tsai-partington test. Yerkes-Dodson Law [83] suggests that there is an inverted-U shape relationship between performance and arousal, the optimal arousal level corresponds to an optimal performance level. Arousal could play a crucial role in performance, but it remains unclear why some tests were affected with other tests showed no effect. The two tests which found significantly improved performance with quicker response at elevated CO_2 levels were the Selective Attention test (SAT) and the Progressive Ratio test (PRT). SAT mainly assessed individuals' attention and PRT focused on motivation and concentration. Therefore, the improvement of performance measured by response times in the two tests could be due to higher arousal under higher CO_2 levels. None of the ten BARS tests found statistically significant effects on the error rate at elevated pure CO_2 levels, consistent with findings in most studies [19–23,26] that examined the effects of added CO_2 under fixed ventilation rates.

Another explanation of the significant results of response time in two tests could be increases in alpha error due to multiple comparisons [84,85] of ten tests. Significant results from numerous tests sometimes could be false positives due to chance, i.e., a significant outcome even if no real effect exist. A number of methods [86] have been developed to correct for alpha error, however, there is no universally accepted standard method on this problem [85]. Bonferroni has been used as a classical method, and p-value adjusted after Bonferroni correction should be 0.005 for this study. In response to the adjusted value, only the Selective Attention test results remained significant and showed significant improvement at 2100 ppm compare to 600 ppm (p = 0.002). Bonferroni is frequently claimed to be excessively conservative [87], increasing the chance of beta error or requiring to increase sample size [85].

Compared with the baseline condition, the present results only found

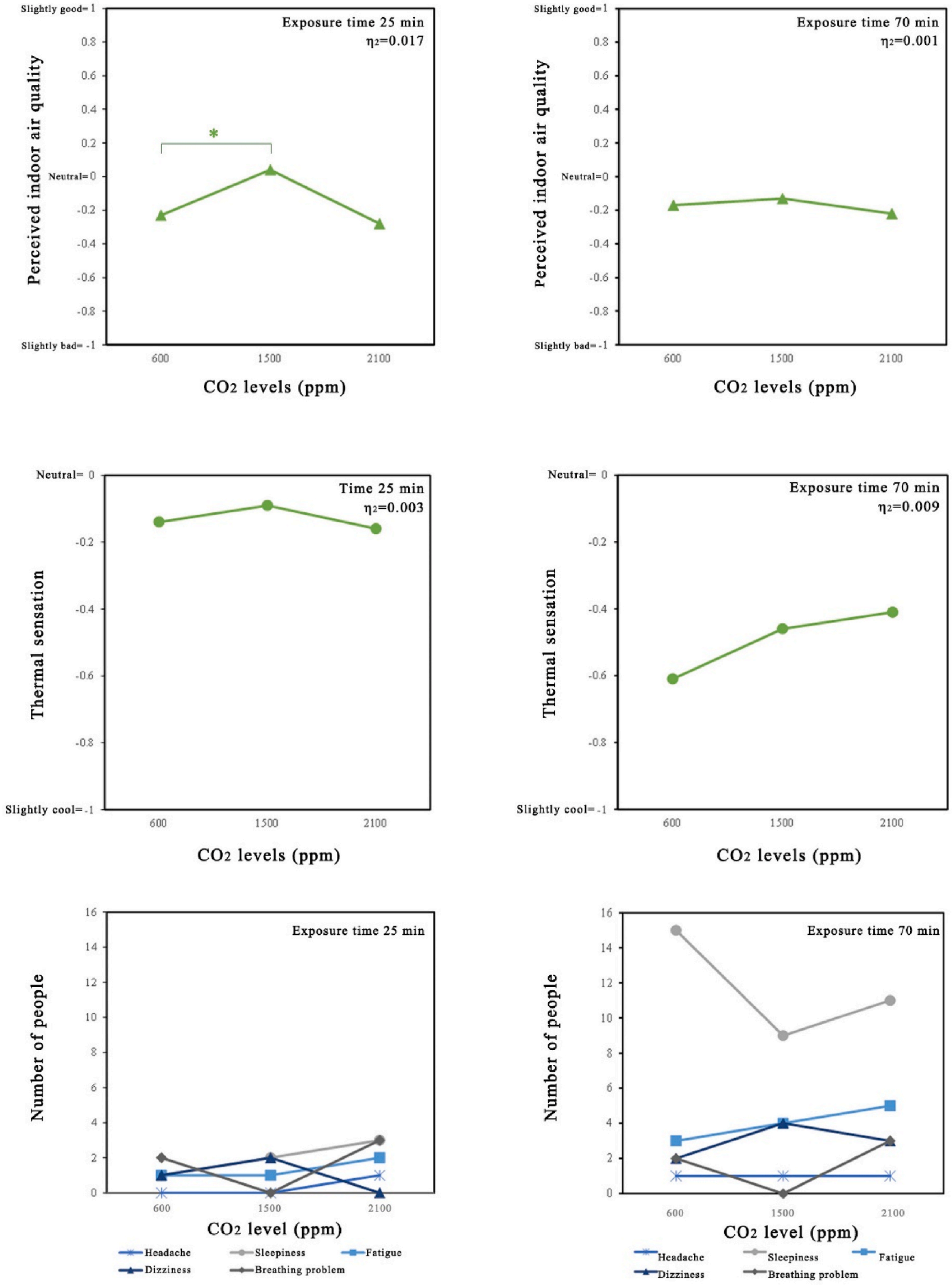

Fig. 5. Subjective assessment of perceived air quality, thermal sensation and number of people reported health symptoms before and after the BARS test (measured at 25min and 70min) at three CO_2 conditions. $^{*}p < 0.05$.

a significant increase in subjective ratings of perceived air quality before the BARS test at 1500 ppm, but no differences at 2100 ppm. Even though the mean values of ratings at intermediate levels statistically significantly varied from 600 ppm, the effect size ($\eta^2 = 0.017$) indicated a small effect. No effects were reported on the ratings of perceived air quality after the BARS test. The results provide little evidence of discrepancies in the perceived air quality at increased CO_2 concentrations. No significant differences were found in other perceived indoor environment qualities (thermal sensation, perceived lighting quality, perceived noise level) and acute health symptoms (headache, sleepiness, dizziness, fatigue and breathing difficulty), and all effect sizes below 0.01 indicated a small effect. These findings were consistent with previous studies that reported no significant results on the perceived indoor environment quality and physiological symptoms at CO_2 levels below 2500 ppm (Table S1).

This study showed several strengths: (1) use of fixed high ventilation rates and added pure CO_2 to reach defined exposure concentrations, which isolates the effect of CO_2 from other indoor air pollutants; (2) BARS test battery was a validated and sensitive computerised neurobehavioral test battery; (3) parallel tests generated by BARS test battery and balanced order of the test sequence could minimize the practice effect; (4) participants were tested alone in the chamber in each experiment session, which could minimize the impact from other participants present during the exposure; (5) the environmental chamber where the study conducted demonstrated highly precise automatic control of the defined indoor environment conditions, reducing the confounding factors; (6) the long intervals of at least four weeks between two experiment sessions for each participant, to minimize any carryover effect from one condition to the later condition, especially the practice effect of cognitive tests; (7) confounding factors were controlled in an experimental design and the statistical analysis to focus on the association of CO_2 with cognitive performance.

Some limitations of this study should be noted and improved in future studies. Firstly, the exposure duration of 70 min might not be long enough for pure CO_2 below 2100 ppm to exhibit effects on people, as how long it takes for moderate CO_2 concentrations to take effects in humans still remains unclear. Secondly, the choice of the study population is limited to a specific group. Participants were all university students aged 25 ± 4 years. Therefore, the results can only represent and reflect the effects of CO_2 in a small part of populations in the educational environment. Thirdly, although the BARS test is a systematically constructed battery based on many validated classical neurobehavioral tests, it was designed mainly to assess neurotoxic chemical exposure such as pesticides and toluene. The null effects might be due to the CO_2 concentration levels tested being within building guidelines and probably not neurotoxic for people. Compared with other batteries used in previous studies such as SMS [14,15,18,31], Cognition [31], CNS Vital signs [27] and Cognitive Drug Research [12], the BARS battery only covers part of the cognitive domains vital to people's daily cognitive function, domains such as reasoning [31], decision-making [14,15], abstraction [31] and emotion cognition [12,31] were not measured. An ideal future study could combine assessments for specific tasks related to participants' professional requirements with reliable general cognitive measures. Fourthly, the original sample size calculation did not consider the possible false positive increase due to the ten tests employed in the experiment but were analysed separately. Alpha error adjusted after Bonferroni correction should be 0.005 (0.05/10), and the estimated sample size would be 93 participants (details of sensitivity analysis in Fig. S2, Supplementary Material). The sample size of 69 used in this study, corresponded to an alpha error of 0.025, which means the present sample size may increase the possibility of false positives. Lastly, there is one limitation with TVOC concentrations measured by Tiger sensor in this study. Tiger sensor with PID used isobutylene (2-methylpropene, C_4H_8) as calibration gas so TVOC level here was equivalent concentration of calibration gas. In the Building Regulations, TVOC is defined as a chromatographis signal for vapors (VOCs) between C_6 and C_{16}. The measured concentrations in this study cannot be compared with building standard such as Ventilation: Approved Document F (2021) [80] which referred to 300 $\mu g/m^3$ of TVOC.

5. Conclusion

This study examined whether different concentrations of carbon dioxide affect people's cognitive performance, perceived indoor environment quality and acute self-reported health symptoms, independent of ventilation rates, within the range suggested by educational building ventilation guidelines. Performance significantly improved at elevated CO_2 levels in Progressive Ratio and Selective Attention tests which measured motivation and sustained attention, possibly due to impact of higher arousal. With only two out of ten tests showing significant decreases in response times and no significant results in accuracy performance under higher CO_2 levels, the findings indicated no clear links between exposures of pure CO_2 up to 2100 ppm for 70 min and cognitive domains of memory, attention, motor speed, coordination, and learning in university students, compared to a baseline of 600 ppm. Elevated CO_2 was not associated with acute health symptoms and perceived indoor environment quality, except for limited evidence in perceived air quality. These findings provide empirical evidence that the cognitive performance of university students, measured by the BARS test battery, was not adversely affected by pure CO_2 at levels routinely experienced in the educational building environment, consistent with the current guidelines and some previous studies. More importantly, not only that some of the results from prior studies which reported decrements in cognitive performance could not be confirmed in this study, the contrary was found. This adds to the uncertain nature of evidence in this field, although more research is needed to confirm this finding which could be due to arousal effects or to numerous testing across several variables. The results indicate that pure CO_2 levels below 2100 ppm should not be treated as a potential indoor pollutant on its own, while concerns still exist when levels rise above that to the occupational exposure limit of 5000 ppm. Future research should incorporate individuals from other populations along with cognitive assessment methods that imitate the specific learning/working process, may contribute to a better understanding of how elevated CO_2 affects cognitive performance.

CRediT authorship contribution statement

Didong Chen: Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Resources, Visualization, Writing – original draft. **Gesche Huebner:** Validation, Supervision, Writing – review & editing. **Emmanouil Bagkeris:** Software, Formal analysis. **Marcella Ucci:** Validation, Supervision, Writing – review & editing. **Dejan Mumovic:** Validation, Supervision, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.buildenv.2023.110967.

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